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# ENVIRONMENTAL QUALITY RESEARCH -- FATE OF TOXIC JET FUEL COMPONENTS IN AQUATIC SYSTEMS

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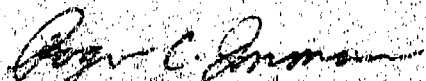
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This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER



ROGER C. INMAN, Colonel, USAF  
Chief  
Toxic Hazards Division  
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## SUMMARY AND CONCLUSIONS

Development work continued in the earlier part of the year on our jet fuel bioassay procedure involving the effect on the hatchability of brine shrimp (Artemia salina) eggs. Artemia hatching efficiencies were increased 10-fold in saline (1.6 %) relative to fresh water. The hatch was also improved substantially by using ASTM Type I water instead of 'regular' laboratory distilled water.

Decapsulation (dechoriation) of Artemia eggs by treatment with sodium hypochlorite improved the hatch - to > 90 % with some egg batches.

Hydrocarbon storage losses from water soluble extracts (WSF) of the fuels were found to be due to biodegradation. They could be virtually eliminated by limiting WSF preparation time to 48 hours and the subsequent bioassay to 48 hours.

Reduced dissolved oxygen (DO) levels (3.7 mg/L), well below the levels reached in our closed bioassay system, had little effect on hatching efficiency.

The protocol for the Artemia Hatchability Bioassay was finalized. Dechorionated eggs in batches of 100 were exposed to various WSF dilutions for 48 hours at 26°C. LC 50 values were calculated from the hatch counts using the moving average method. A computer program was written to facilitate the computation.

Improvements in our gas chromatography procedure for analyzing neat fuel and WSF hydrocarbons were achieved by switching to a programmable Hewlett Packard 5840A GC, using a different internal reference standard (n-C<sub>16</sub>) to reduce interference with WSF peaks and calibrating the system for an increased range of individual hydrocarbons, both aromatic and aliphatic.

The reduction in JP-4 water solubilities associated with the use of saline solutions (for the Artemia bioassay) was found to be minor (10-15 %).

Sparging tests with N<sub>2</sub> and air on WSF's demonstrated a rapid loss of hydrocarbons (80 % in 2 minutes) and a nearly equivalent loss of toxicity.

JP-4 samples from five different suppliers, including an experimental shale-derived sample, were compared by GC as neat fuels and WSF's. Although most had similar components there were substantial differences in their relative amounts. The major components in all the WSF's were the aromatics: benzene, toluene, and the xylenes.

The water solubilities (1.6 % salinity) and toxicities of a number of individual hydrocarbons present as major or minor components in JP-4 WSF's were determined. Benzene and aromatics in general were substantially less toxic than the alkanes. However, their much higher solubility and therefore higher dose level may allow them to exert comparable or greater toxic effects.

The toxicities of the five different JP-4 samples were found to vary over a 3-fold range. The shale-derived sample was least toxic when LC50 was expressed as percent WSF but most toxic in terms of total hydrocarbons.

Although benzene, toluene, and the xylenes comprise 70-90 % of the total WSF hydrocarbons, because of their low toxicity they contribute < 20-30 % to the total WSF toxicity.

Tests with 'simulated' JP-4 mixtures of the major WSF hydrocarbons, both aromatic and alkanes, gave no indication of synergistic effects that might explain the high JP-4 toxicity despite the low (major) component toxicity.

The toxicity of the 'residual' WSF hydrocarbons needed to account for the overall WSF toxicity in the light of the 'small' contribution by the major aromatics was estimated to be ~ 2 ppm. This is close to the actual toxicity of alkanes such as n-pentane and n-hexane and therefore supports the hypothesis that the unaccounted-for WSF toxicity is due at least in substantial part to the aliphatic components.

The toxicity of JP-4 samples can be predicted approximately from the amounts of benzene and toluene in the neat fuel.

WSF toxicity can also be predicted, somewhat more accurately, from a GC analysis of the total dissolved hydrocarbons, thus avoiding the inevitable delays inherent in bioassay procedures.

Provided the appropriate individual component toxicities are known or can be determined, the Individual Toxic Contribution of a Hydrocarbon (ITCH No.) to the overall WSF toxicity can be calculated. Representative ITCH numbers for major WSF hydrocarbons are benzene 2-10 %, toluene 6-13 %, xylenes 6-20 %, n-pentane 2-10 %.

A brief attempt was made to clarify the relationship between the toxic contributions of aromatic and aliphatic hydrocarbons and the actual toxicity of the JP-4 WSF's.

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of total) accounted for less than 30 % of WSF toxicity; and 3) the estimated toxicity of the remaining WSF hydrocarbons was high enough (~ 2 ppm) to account for the rest of the WSF toxicity. It was concluded that all the JP-4 hydrocarbons were toxic, their contribution being dependent on the proportion present in the water soluble fraction, and that JP-4 toxicity was the sum of the toxicities of its component hydrocarbons. Least squares plots have been developed that allow prediction of maximum JP-4 WSF toxicities from 1) benzene/toluene levels in the neat fuel and 2) total hydrocarbon levels in the WSF.

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## PREFACE

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## INTRODUCTION

The primary goal of the Berkeley—SEEHRL contract is to investigate the fate of toxic fuel components in the aquatic environment. Specifically, we have been investigating the sources of the toxic activity of the jet fuel JP-4.

At the outset it was thought likely that it would be necessary to carry out a careful fractionation (by distillation, chromatography, etc.) of the fuel in order to segregate, recover, and hopefully identify the toxic entities. When preliminary work indicated that the water soluble extract (WSF) of JP-4 consisted of (in addition to many minor components) only a few major, mainly aromatic, hydrocarbons readily identified as benzene, toluene, and the xylenes, it was felt that the presence of these compounds, widely regarded as primary toxicants in petroleum fractions, might very well account for all the JP-4 toxicity. When this proved not to be the case, these materials contributing only a relatively small fraction of the total JP-4 toxicity, it again appeared possible that there might be a limited number of major toxicants, not necessarily hydrocarbon in nature, in the fuel. Before starting to search for non-hydrocarbon species the possibility of synergistic effects in the complex mixture of hydrocarbons that comprised the jet fuel and, to a lesser extent, the WSF was investigated. Toxicity measurements were made on a range of aromatic and saturated hydrocarbons and cycloparaffins and predicted and measured toxicities were compared for synthetic mixtures of known composition. These experiments indicated that synergistic effects were probably minimal and that the JP-4 toxicity could be explained on the basis of its hydrocarbon content and the somewhat unexpected findings on the relative toxicities of the different hydrocarbon classes.

Samples of JP-4 from different sources have been compared for toxicity and composition. This work was designed to investigate the variability of the fuel, an important factor in deciding whether generalizations regarding toxicity, etc. on the basis of a single fuel sample were valid. When differences were indeed found the possibility of utilizing them to help predict JP-4 water solubilities and toxicities was realized and successfully demonstrated.

## BIOASSAY DEVELOPMENT

During the present year our bioassay efforts have centered on the Artemia salina hatchability test for which the preliminary work was reported in the Annual Report for 1980/1981 (Cooper et al., 1981). During the earlier part of the present period a number of variables affecting the test were investigated. These included hydrocarbon storage losses, salinity effects, dissolved oxygen requirements, and the advantages of, and procedures for, egg decapsulation (dechoriation).

### HYDROCARBON STORAGE LOSSES

In the Cooper et al. (1981) report we noted that substantial losses (60-70%) of JP-4 water-soluble extract (WSF) hydrocarbons appeared to take place during the 2-3 day period of a bioassay run. Since the losses occurred even in the presence of  $\text{HgCl}_2$ , it was thought that biodegradation was not the cause. One alternative explanation was adsorption onto the glass container surfaces. Subsequent investigations have indicated that this is not the case and that biodegradation very probably was involved, although we have never been able to reproduce the large losses of the earlier reported experiments. In one series of tests the use of different test bottle cleaning procedures was examined since this might very well influence adsorptive effects. The WSF was prepared as described by Cooper et al. (1981). Cleaning procedures included a) soap-and-water wash and rinse and b) an additional acetone rinse. The bottles were allowed to drain dry overnight. In a second test the bottles were oven-dried at  $105^\circ\text{C}$ . All bottles were filled with WSF and stored tightly sealed for 72 hours at  $27^\circ\text{C}$ . Samples were analyzed by gas chromatography before and after storage. The results (Table 1) showed no significant difference between treatments but more surprisingly there were no significant hydrocarbon losses even after 72 hours of storage. These results were supported by the essentially unchanged WSF hydrocarbon concentrations at 0 hour and 48 hours in the controls for the various bioassay runs of which Table 2 is a typical example.

Although the storage loss problem seemed to have disappeared, it was felt desirable to investigate it further to avoid a possible recurrence. One possible source of trouble was the WSF preparation, involving prolonged

TABLE 1  
EFFECT OF BOTTLE TREATMENT ON WSF HYDROCARBON LEVELS

	Dried at Room Temp.				Oven Dried (105° C)			
	72 hours Storage		0 hour Storage <sup>a</sup>		72 hours Storage		S/W Wash Acetone Rinse	
	0 hour Storage <sup>a</sup>	S/W <sup>b</sup> Wash	S/W Wash-Acetone Rinse	0 hour Storage <sup>a</sup>	S/W Wash	S/W Wash Acetone Rinse	S/W Wash	S/W Wash-Acetone Rinse
	ppm w/v ± S.D.	ppm w/v ± S.D.	ppm w/v ± S.D.	ppm w/v ± S.D.	ppm w/v ± S.D.	ppm w/v ± S.D.	ppm w/v ± S.D.	ppm w/v ± S.D.
Benzene	11.9±0.6	12.25±0.8	12.85±0.07	11.8±0.7	12.1±0.1	11.9±0.42		
Toluene	8.45±0.35	8.4±0.7	8.65±0.07	8.3±0.3	8.55±0.07	8.6±0.3		
Xylenes	3.45±0.07	3.4±0.2	3.55±0.01	4.2±0.2	4.4±0.08	4.4±0.1		
Peak "A" <sup>c</sup>	0.455±0.035	0.455±0.02	0.44	0.47±0.03	0.475±.007	0.475±0.04		
Total Hydrocarbons	31.05±0.07	30.1±2.3	31.4±0.14	34.1±2.6	35.65±0.21	35.2±1.1		

<sup>a</sup> The WSF for each series was prepared separately.

<sup>b</sup> S/W = Soap/Water

<sup>c</sup> Peak A is small alkane peak emerging just ahead of the benzene peak.



TABLE 2  
EFFECT OF STORAGE ON WSF HYDROCARBONS IN  
BIOASSAY CONTROL SAMPLE  
(ppm w/v)

	Storage Period	
	0 Hour	48 Hours
Benzene	10.75	10.9
Toluene	7.4	7.4
Xylenes	3.39	3.36
Peak "A" <sup>a</sup>	0.395	0.40
Total Hydrocarbon	28.5	28.9

<sup>a</sup> Peak A is small alkane peak emerging just ahead of the benzene peak.

contact of water and fuel phases. In our early experiments before minimum times for satisfactory WSF preparation had been established the contact period was 72 hours. Later 24 hours was shown to be adequate. It seemed possible that the earlier extended period might have allowed microorganisms in the Richmond Field Station well water to adapt to the aqueous JP-4 components. This point was tested in duplicated experiments using "72-hour" WSF's. In both test series parallel tests were run with de-ionized water. The results (Table 3) indicated that significant losses occurred in the well water but not in the de-ionized water. For example, losses as high as 25% and 50% were found for toluene and ethylbenzene in both test series. The much smaller losses in the de-ionized water samples are probably within the reproducibility of the analysis. Alternatively, the relative constancy of these losses for different hydrocarbons suggests mechanical losses during the experiment rather than biodegradation. Thus these experiments supported the existence of a biodegradative loss mechanism in the well water WSF although the losses were substantially below those found in the earlier tests. A possible explanation for this is variability in the micro-organism population of the well water at different times of the year.

Since the losses were at most minimal using de-ionized water and would be further reduced by shortening the WSF preparation time to 24

TABLE 3  
EFFECT OF STORAGE ON JP-4 WSF HYDROCARBONS

	Series I						Series II					
	Deionized H <sub>2</sub> O			RFS Well H <sub>2</sub> O			Deionized H <sub>2</sub> O			RFS Well H <sub>2</sub> O		
	0 hr	72 hr <sup>a</sup>	Loss %	0 hr	72 hr	Loss %	0 hr	72 hr	Loss %	0 hr	72 hr	Loss %
	ppm w/v	ppm w/v		ppm w/v	ppm w/v		ppm w/v	ppm w/v		ppm w/v	ppm w/v	
Benzene	13.4 ± .004 <sup>b</sup>	13.0	3.3	13.3 ± .07	12.4 ± .004	6.4	13.2 ± .49	13.1 ± .016	0.8	13.3 ± .11	12.4 ± .007	6.8
Toluene	9.7 ± .004	9.3	4.1	9.8 ± .14	7.6 ± .004	22.4	9.4 ± .14	9.2 ± .011	2.1	9.5 ± .06	6.8 ± .09	28.4
Ethylbenzene	0.77 ± .001	0.73	5.2	0.81 ± .02	0.30 ± .004	62.7	0.78 ± .0 <sup>+</sup>	0.76 ± .0001	2.6	0.76 ± .006	0.36 ± .008	52.6
Xylenes (o-, m-, and p-)	3.96 ± .003	3.74	5.6	4.02 ± .0004	3.2 ± .0004	17.4	3.85 ± .04	3.88 ± .0007	+0.8	3.86 ± .01	3.88 ± .03	+0.5
Peak A	0.52 ± .007	0.48	6.8	0.52 ± .007	0.48 ± .007	7.7	0.50 ± .014	0.48 ± .008	4.0	0.50 ± .004	0.49 ± .002	2.0
Total HC	33.3 ± .004	31.8	4.5	34.4 ± .141	28.1 ± .14	18.3	32.7 ± .35	31.7 ± .07	3.1	33.0 ± .92	28.3 ± .04	14.2

<sup>a</sup> Single analysis

<sup>b</sup> ± 1 standard deviation

hours it was felt that they would not affect the Artemia Hatching Bioassay to any significant degree.

#### SALINITY EFFECTS

Initially we had hoped to conduct the Artemia Hatchability Tests in freshwater, based on the prior assumption that this might be possible. However, in view of the very low (5%) control hatching efficiencies in preliminary tests, reported in last year's Annual Report (Cooper et al., 1981), a series of hatching tests was performed at different salinity levels and with the salinity supplied by a) natural seawater, b) rock salt, and c) "Instant Ocean" (Aquarium Systems, Mentor, Ohio). In general, these tests involved incubating brine shrimp eggs in batches of several hundred in closed (to simulate the necessary closed system for bioassaying jet fuel water extracts), completely filled glass containers (130 mL) for several days at a known temperature ( $\sim 27^{\circ}\text{C}$ ). The seawater was filtered and UV-sterilized before use. The nauplii were counted at fixed time intervals. The results of the first test series evaluating the effect of different (seawater) salinity levels are given in Table 4 and plotted in Figure 1. The beneficial effects of increasing salinity on hatching efficiency are clear. Furthermore, hatching is essentially complete in 48 hours at the higher salinity levels (1.6% and 3.2%).

Since it would be much more convenient to use rock salt or "Instant Ocean" to produce the desired salinity additional tests were run (subsequent to the decapsulation tests described in the next section) substituting these components for seawater. Table 5 and Figure 2 summarize the results. With rock salt hatchability, never very high, actually decreased at levels above 1%. "Instant Ocean" on the other hand gave comparable (75%) hatching efficiencies to seawater. The hatch apparently dropped off at concentrations above 2%, possibly due to the presence of a precipitate that formed at 3%. Subsequently, excellent hatchabilities were found up to 3% salinity levels when the precipitated (or undissolved) material was filtered off prior to testing. In these same latter tests comparisons between ASTM Type I water and our regular laboratory distilled grade revealed the superiority of the former, especially in producing a more rapid hatch (Table 6).

TABLE 4  
EFFECT OF SEAWATER SALINITY ON HATCH EFFICIENCY  
OF Artemia salina EGGS

Conditions: 200 eggs per test, incubated at 27°C, continuous light

Salinity %	48-hour Test Period		72-hour Test Period	
	Nauplii	Hatch, %	Nauplii	Hatch, %
0 A	3	1.5	11	5.5
0 B	3	1.5	8	4.0
0 C	6	3.0	12	6.0
0.8 A	15	7.5	22	11.0
0.8 B	14	7.0	18	9.0
0.8 C	20	10.0	31	15.5
1.6 A	25	12.5	27	13.5
1.6 B	35	17.5	36	18.0
1.6 C	33	16.5	36	18.0
3.2 A	36	18	36	18.0
3.2 B	44	22	47	23.5
3.2 C	40	20	42	21.0

TABLE 5  
EFFECT OF WATER SOURCE ON Artemia HATCHABILITY  
IN "INSTANT OCEAN"

Conditions: 100 eggs per test, incubated at 26 ± 1°C  
Hatch, %

Salinity %	ASTM Type I Water		Distilled Water	
	24 hour	96 hour	24 hour	96 hour
1.5	52	63	1	54
	56	60	4	45
2.0	ND <sup>a</sup>	ND	ND	ND
3.0	70	82	ND	ND
	64	74		

<sup>a</sup> ND = not determined

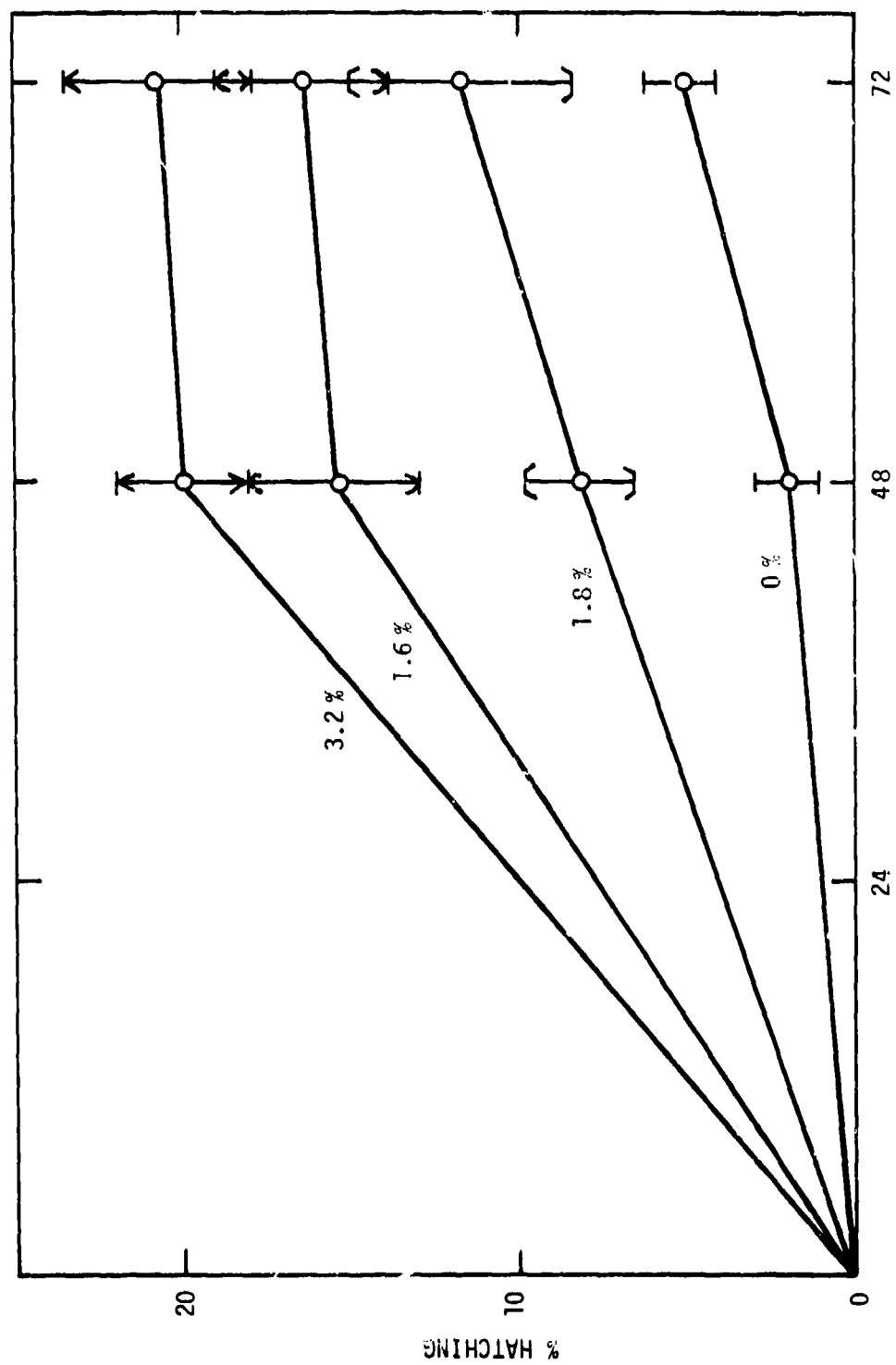


FIGURE 1. EFFECT OF SALINITY (SEAWATER) ON *Artemia* HATCHING EFFICIENCY (AT 27°C UNDER CONTINUOUS LIGHT)  
(Values are mean  $\pm$  one standard deviation.)

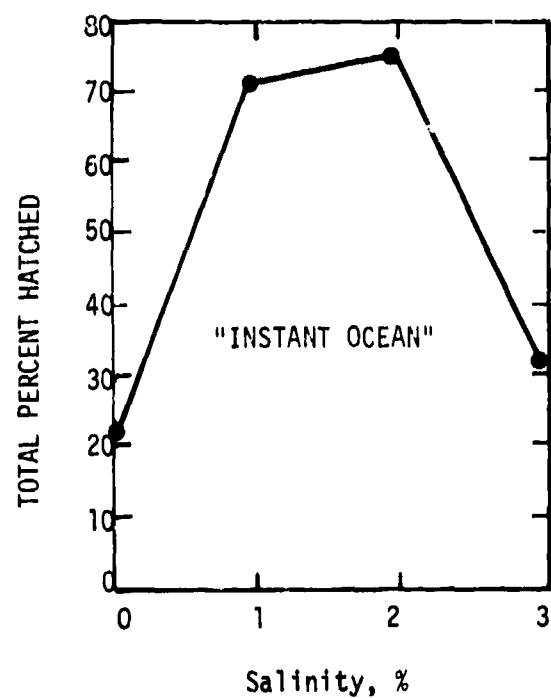
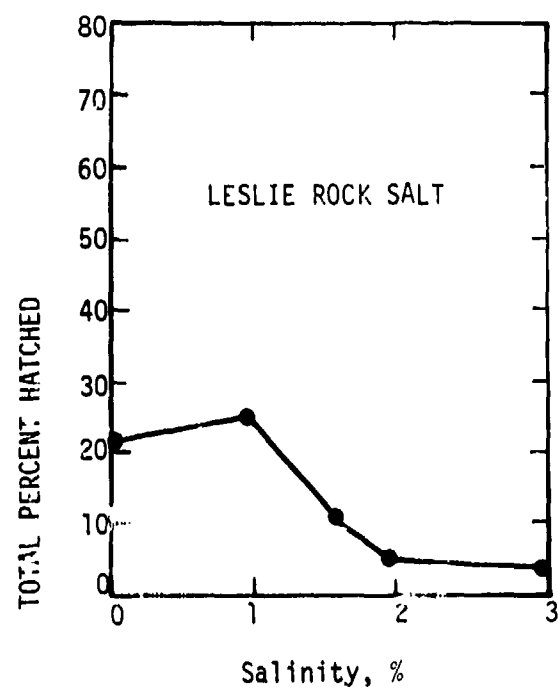


FIGURE 2. COMPARISON OF THE EFFECT OF ROCK SALT (LESLIE) AND "INSTANT OCEAN" ON Artemia HATCHABILITY  
(Based on 48-hr mean values from Table 4)

TABLE 6

Artemia HATCHING EFFICIENCY IN ROCK SALT  
AND "INSTANT OCEAN" SOLUTIONS

Conditions: 100 eggs per test, incubated at  $26 \pm 1^\circ\text{C}$

Salinity %	Series I <sup>a</sup>		Series II <sup>a</sup>	
	24 hr Nauplii Count (Hatch %)	48 hr Nauplii Count (Hatch %)	24 hr Nauplii Count (Hatch %)	48 hr Nauplii Count (Hatch %)
0	0	19	14	28
0	13	19	17	23
1	0	20	0	23
1	1	28	0	24
Rock Salt (Leslie)	1.6	0	0	5
	1.6	7	0	7
	2.0	6	0	4
	2.0	5	0	5
	3.0	4	0	5
	3.0	3	0	4
1.0	16	75	4	70
1.0	23	68	12	72
"Instant Ocean"	2.0	6	8	75
	2.0	11	2	78
	3.0	2	0	26
	3.0	2	0	37

<sup>a</sup>

Series differed only in prehydration conditions (Series I: 20 hours at  $9^\circ\text{C}$ ; Series II: 1 hour at  $25^\circ\text{C}$ ) before decapsulation.

From these experiments it was concluded that satisfactory hatching levels in the range of 60–80% could be achieved using "Instant Ocean" at a concentration of 1.6% and incubating for a period of 48 hours at  $26\text{--}27^\circ\text{C}$ .

## DECHORIONATION

The advantages of dechoriation (decapsulation) of brine shrimp eggs in achieving faster hatching and higher hatching efficiencies have been noted by Sorgeloos et al. (1977). Since our hatching efficiencies even under saline conditions were still a disappointingly low 20% (Table 4), we decided to try dechorionated eggs in our assay. The decapsulation was based on a method described by Sorgeloos et al. After a prehydration period of 1 hour the cysts were treated with a concentrated sodium hypochlorite-sodium hydroxide solution (200 mL bleach, 100 mL H<sub>2</sub>O, 7.5 mL NaOH), initially for 3.5 minutes. The dechorionated eggs were rinsed with water for 1 hour. Hatching tests were then conducted on batches of the eggs over a range of salinities. Nauplii counts were made at 24 hours and 48 hours (an additional 72-hour count was made on the 0% salinity control). The results shown in Table 7 and Figure 3 confirm the substantial improvement in hatching efficiencies with values as high as 70-90% after 48 hours. Further tests confirmed the high hatch efficiency and indicated that:

1. Sodium hydroxide was not required for the dechoriation;  
adequate basic conditions are provided by the hypochlorite alone.

TABLE 7

### HATCHABILITY OF DECHORIONATED Artemia CYSTS AT DIFFERENT SALINITY LEVELS

Conditions: ~400 eggs per test, incubated at 27°C under continuous light

<u>% Salinity</u>	<u>24 hr</u>	<u>% Hatch- ability</u>	<u>48 hr</u>	<u>% Hatch- ability</u>	<u>72 hr</u>	<u>% Hatch- ability</u>
0 A	0	0	9	2.2	38	9.4
0 B	1	0.2	10	2.5	45	11.1
0.8 A	14	3.4	294	72.4	-	-
0.8 B	12	3.0	341	84.0	-	-
1.6 A	306	75.4	369	90.9	-	-
1.6 B	291	71.7	357	87.9	-	-
3.2 A	313	77.0	347	85.5	-	-
3.2 B	348	85.7	389	95.8	-	-



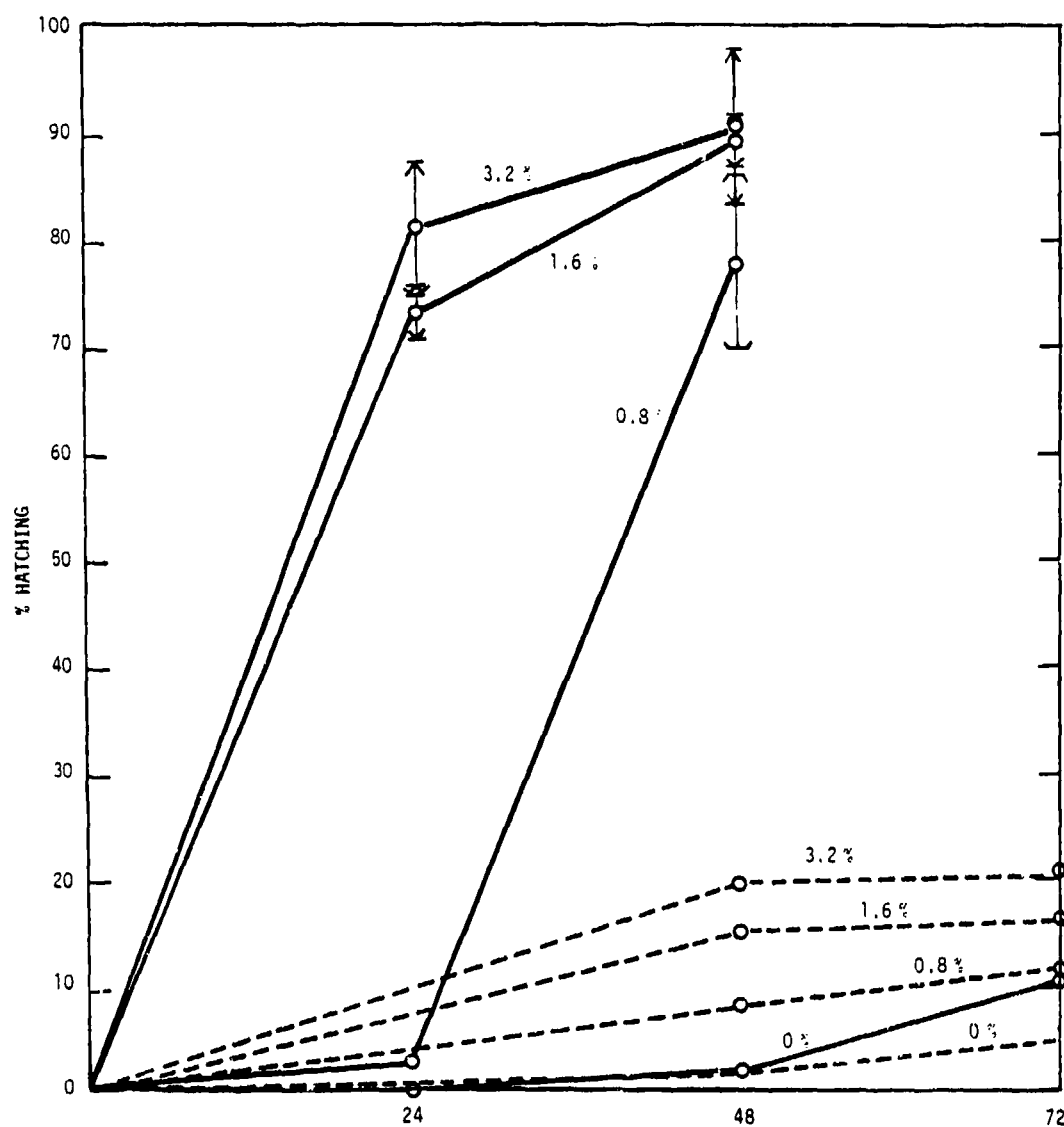


FIGURE 3. EFFECT OF DECHORIONATION ON *Artemia* HATCHING EFFICIENCY AT 27°C AND DIFFERENT SALINITIES (Values are mean  $\pm$  1 S.D.; solid lines = dechorionated eggs; dashed lines = untreated eggs [based on Figure 1].)

2. Continuous lighting was not necessary and did not appear to increase the hatchability of the dechorionated eggs.
3. The hypochlorite exposure time was not critical, at least over a range of 3 to 6 minutes.

On the basis of these tests it was decided to include a cyst dechoriation step as a standard feature of our Hatchability Bioassay Test.

#### DISSOLVED OXYGEN (DO) LEVELS

In order to prevent the loss of volatile fuel components it is necessary to conduct our Artemia Hatchability Bioassay in closed, completely-filled containers. Although past experience (Cooper et al., 1981) had indicated relatively slight reduction in stored WSF samples, it was desirable to establish just how critical the DO level was to Artemia hatchability. Therefore, a series of tests were run under the optimum conditions established as described above, i.e., using filtered 1.6% "Instant Ocean" solution in ASTM Type I water (but containing no WSF). The DO level in different containers was adjusted by a nitrogen purge to the values listed in Table 8. One hundred dechorionated eggs were incubated in each container for 96 hours at  $26 \pm 1^\circ\text{C}$ . The results are summarized in Table 8 and Figure 4. They indicate that DO levels down to  $\sim 3.7$  mg/L – well below any levels encountered in our Artemia bioassay – have no appreciable effect on hatchability.

TABLE 8  
EFFECT OF DISSOLVED OXYGEN LEVEL ON Artemia salina HATCHABILITY  
Conditions: 100 eggs per test, incubated at  $26 \pm 1^\circ\text{C}$  for 96 hours

Test Series I		Test Series II	
DO Level mg/L	Nauplii Count <sup>a</sup> (Hatch %)	DO Level mg/L	Nauplii Count <sup>a</sup> (Hatch %)
7.2	100 <sup>b</sup>	7.2	100 <sup>b</sup>
5.3	91	2.3	43
3.7	96	1.8	47
2.4	71	1.1	6
0.7	9	0.5	5

<sup>a</sup> Duplicate tests run at each level

<sup>b</sup> Hatch at this (highest) DO level assigned a value of 100%

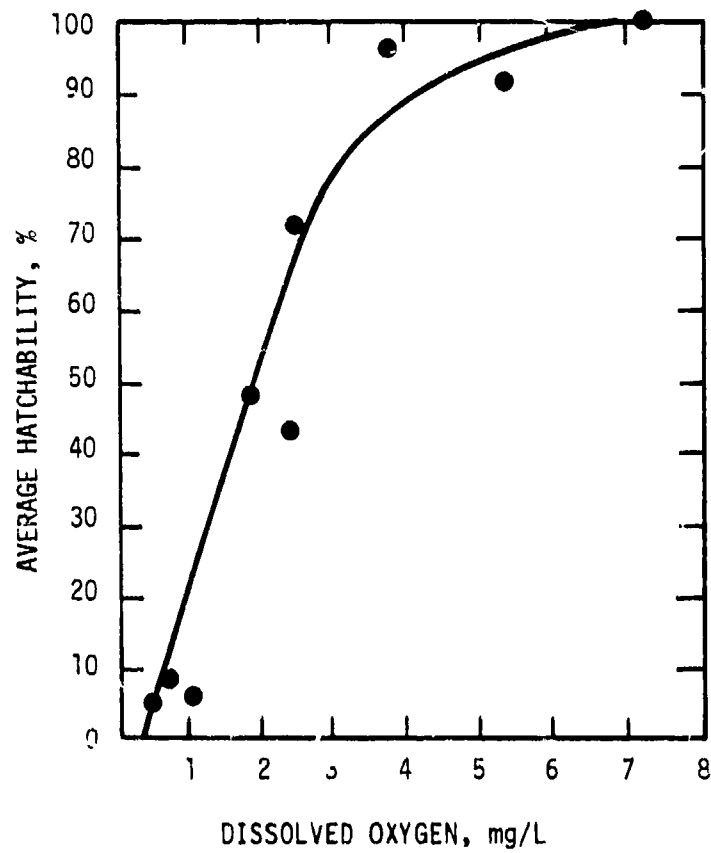


FIGURE 4. EFFECT OF DISSOLVED OXYGEN ON HATCHABILITY OF Artemia CYSTS

## JP-4 INVESTIGATIONS

### CHEMICAL AND PHYSICAL COMPARISONS

#### Analytical Methods

The basic extraction and gas chromatographic procedures for the analysis of hydrocarbons in neat fuels and their water soluble extracts (WSF's) were described in last year's report (Cooper et al., 1981). Subsequently, we have switched to a more modern Hewlett Packard Model 5840A gas chromatograph which has many advantages accruing from its flexible programmability, not the least its ability to calibrate automatically and retain the calibrations for future analyses. We now use n-hexadecane as our internal reference standard instead of n-pentadecane since there are fewer and smaller interfering peaks (either from the WSF's or septum-column (10% SP-2100 on 80/100 Supelcoport) bleed at the n-C<sub>16</sub> emergence time. With this new chromatograph we have now expanded our calibrations to include (in addition to benzene and toluene) the C<sub>2</sub>H<sub>5</sub> - alkylbenzenes, ethylbenzene, and o-, m-, and p- xylenes and a number of other cyclic hydrocarbons and alkanes including naphthalene, decalin, tetralin, n-pentane, n-hexane, n-heptane, and i-octane. Since we planned to look at the individual toxicities of some of these hydrocarbon components of JP-4 WSF's we have adopted modified procedures for their analysis. Specifically because of reduced peak interference it was possible to use more volatile internal standard hydrocarbons, n-octane or, where appropriate, n-nonane. These standards are more accurate and faster since they emerge close to potential components of interest (benzene, toluene, xylenes, etc.) and in less than half the time for n-hexadecane.

Calibration standards have generally been made up in n-pentane or, where the latter was being analyzed, in n-heptane. However, when the analysis of selected hydrocarbons in neat JP-4's was undertaken the calibration standards were made up in one of the jet fuels (the shale-derived sample) with appropriate corrections for the amount of the analyzed component already present in the fuel. In this manner the hydrocarbon peak areas were calibrated under conditions of peak clustering and overlap comparable to the actual jet fuel analysis and hence were likely to increase the accuracy of the latter.

### Hydrocarbon Water Solubilities

A knowledge of the solubilities of hydrocarbons in water, both as components of jet fuels and as individual hydrocarbons, is essential when one is attempting to identify the toxic components of a fuel since LC 50 values expressed in terms of percent WSF do not indicate the individual toxicities of the components (although they do provide a valid comparison of the overall toxicity of different fuels). Also since we have changed from fresh to salt water to improve the hatch efficiency of the Artemia eggs, the effect of this switch on hydrocarbon solubilities (and hence on toxicity) was of interest.

#### Effect of Salinity Changes .

As mentioned above it was necessary to use salt water as the medium in our Artemia Hatchability Bioassay to improve the eclosion efficiency. It was important to determine whether this modification would affect the jet fuel water solubility enough to cause drastic shifts in toxicity. Therefore a series of WSF's were prepared from JP-4 (GEC-1A-792037) by our standard technique (gentle magnetic stirring for 24 hours at 20°C of the water phase overlaid by the jet fuel) using fresh and salt water, the latter prepared both from rock salt (Leslie, 1.6% and 3.2% concentrations) and from "Instant Ocean" (1.6%). GC analysis of the major hydrocarbons (Table 9) indicated that the reduction in hydrocarbon levels was relatively small (10-15%) at 1.6% salt concentration and hence might be expected to produce detectable but not drastic changes in WSF toxicity. Under such conditions it was considered that the hatching bioassay would still constitute a useful assay system. Although the 1.6% "Instant Ocean" formulation was later selected as the preferred hatching medium on the basis of higher hatch efficiencies, it is interesting to note the apparently greater depressant effect on solubility of the 1.6 % rock salt and the barely significant further decrease on raising the concentration to 3.2 %.

#### Solubility of Individual Hydrocarbons.

For a number of reasons involving their presence as major or minor components in JP-4 WSF samples and their potential contribution to the toxicity of the latter, WSF's of a number of pure hydrocarbons of various types and structures have been prepared for toxicity evaluation. Since it is

TABLE 9  
EFFECT OF SALINITY ON JP-4 WATER SOLUBILITY

	Fresh Water		NaCl				Instant Ocean	
			1.6 %		3.2 %		1.6 %	
	ppm w/v	%	ppm w/v	%	ppm w/v	%	ppm w/v	%
Benzene	12.3	40	10.2	39	10.5	40	11.0	38
Toluene	8.5	28	6.9	26	6.8	26	7.3	25
Ethylbenzene							0.58	
m- + p- Xylenes	4.1	13	3.3	13	3.2	12	2.01	12
o-Xylene							0.91	
Peak A <sup>a</sup>	0.47	1.5	0.36	1.3	0.35	1.3	0.39	1.3
Total HC	30.7	100.0	26.1	100.0	26.1	100.0	28.7	100.0

<sup>a</sup> Small (presumably alkane) peak emerging just before benzene.

meaningless to compare their toxicities on the basis of percent WSF because of their varying water solubilities, the latter have been measured to allow toxicities to be expressed in terms of hydrocarbon concentration by the same procedure used to analyze the JP-4 WSF's, i.e., pentane (or other hydrocarbon as appropriate) extraction followed by direct GC analysis of the pentane concentrate. Since these are individual hydrocarbon WSF's, the resulting numbers approximate to true water "solubilities." The many hydrocarbons comprising the WSF from a hydrocarbon mixture such as a jet fuel are more correctly described as being "equilibrated" as a result of partitioning between the aqueous and fuel phases. The solubility data for all these hydrocarbons have been collected and summarized in Table 10.

#### Effect of Sparging on WSF Hydrocarbon Levels.

As part of our investigation of JP-4 WSF toxicity, the effect of partial removal of hydrocarbons by sparging with nitrogen and air was examined. The sparging was conducted by bubbling the gas through an air-stone into JP-4 (GEC-1A-792033) WSF for varying lengths of time. The treated samples were then analyzed for hydrocarbons by GC in the usual manner. Since the N<sub>2</sub> sparge reduced the DO level substantially, air was substituted

TABLE 10  
SOLUBILITY OF INDIVIDUAL HYDROCARBONS IN WATER AT 20°C

	Deionized Water		1.6 %
	0 hr ppm w/v	24 hr <sup>a</sup> ppm w/v	Instant Ocean ppm w/v
Benzene	1527	1588	944
Toluene	498	498	347 <sup>b</sup>
			332
Ethylbenzene	157	164	121
m-Xylene	162	-	144.5
p-Xylene	154	-	133
o-Xylene	182	-	160
Decalin <sup>c</sup>			
(i) <u>trans</u> -isomer	-	-	0.32
(ii) <u>cis</u> -isomer	-	-	0.54
Tetralin	-	-	33.6 <sup>b</sup>
			34.3
<u>n</u> -Pentane	-	-	33.5 <sup>b</sup>
			33.1
<u>n</u> -Hexane	-	-	9.2
<u>n</u> -Heptane	-	-	2.22
<u>i</u> -Octane	-	-	1.41

<sup>a</sup> 0 hr WSF was re-analyzed after standing for 24 hours to check for changes due to emulsion separation or biodegradation.

<sup>b</sup> Two separate WSF preparations

<sup>c</sup> Mixture of isomers

for the last 2 minutes (or for the full sparge if total time was less than 2 minutes) to reoxygenate the sample for subsequent bioassay tests. (It was considered inadvisable to oxygenate for longer sparge periods because of possible oxidizing effects on the hydrocarbons.)

Two series of tests were run. In the first series, WSF samples were sparged for periods varying from 2 to 32 minutes. Since even the 2-minute sample lost 80% hydrocarbons, a second shorter series was sparged for times ranging from 2 seconds to 128 seconds. The analytical data are given in Table 11 and the figures from the second series plotted in Figure 5.

TABLE II  
EFFECT OF SPARGING ON WSF HYDROCARBON LEVELS

Test Series I	Benzene		Toluene		Ethylbenzene		m- and p-Xylene		o-Xylene		Total Hydrocarbons	
	ppm w/v	%	ppm w/v	%	ppm w/v	%	ppm w/v	%	ppm w/v	%	ppm w/v	%
Spurge time in minutes												
0	11.0±.08	100	7.5±.09	100	0.59±.007	100	2.05±.02	100	1.01±.02	100	26.6±.28	100
2	1.87	17	1.10	15	0.12	20	0.23	26	0.16	15	5.8	22
4	1.63	15	1.00	13	0.08	14	0.52	25	0.154	15	5.4	20
8	0.54	5	0.11	1.5	0.03	6	0.08	4	0.05	5	1.8	7
16	0.08	0.7	0.06	0.8	<.006	<1.0	<0.04	<1.8	<0.02	<1.6	0.9	3
32	ND <sup>a</sup>	-	0.07	0.9	v. small	-	-	-	v. small	-	1.0	4
Test Series II												
Spurge time in seconds												
0	11.4±.07	100	7.1±.14	100	0.57±.004	100	1.97±.002	100	0.96±.004	100	25.9	100
8	10.1	89	5.9	83	0.45	80	1.56	79	0.72	75	21.5	83
16	9.9	87	4.8	67	0.43	77	1.33	67	0.69	72	22.8	88
32	7.9	70	3.9	55	0.33	59	1.06	54	0.57	59	17.7	68
64	6.1	54	2.5	36	0.09	15	0.48	24	0.32	33	-	-
128	4.5	39	1.3	18	0.11	20	0.57	29	0.19	19	9.0	35

<sup>a</sup> ND = not detected



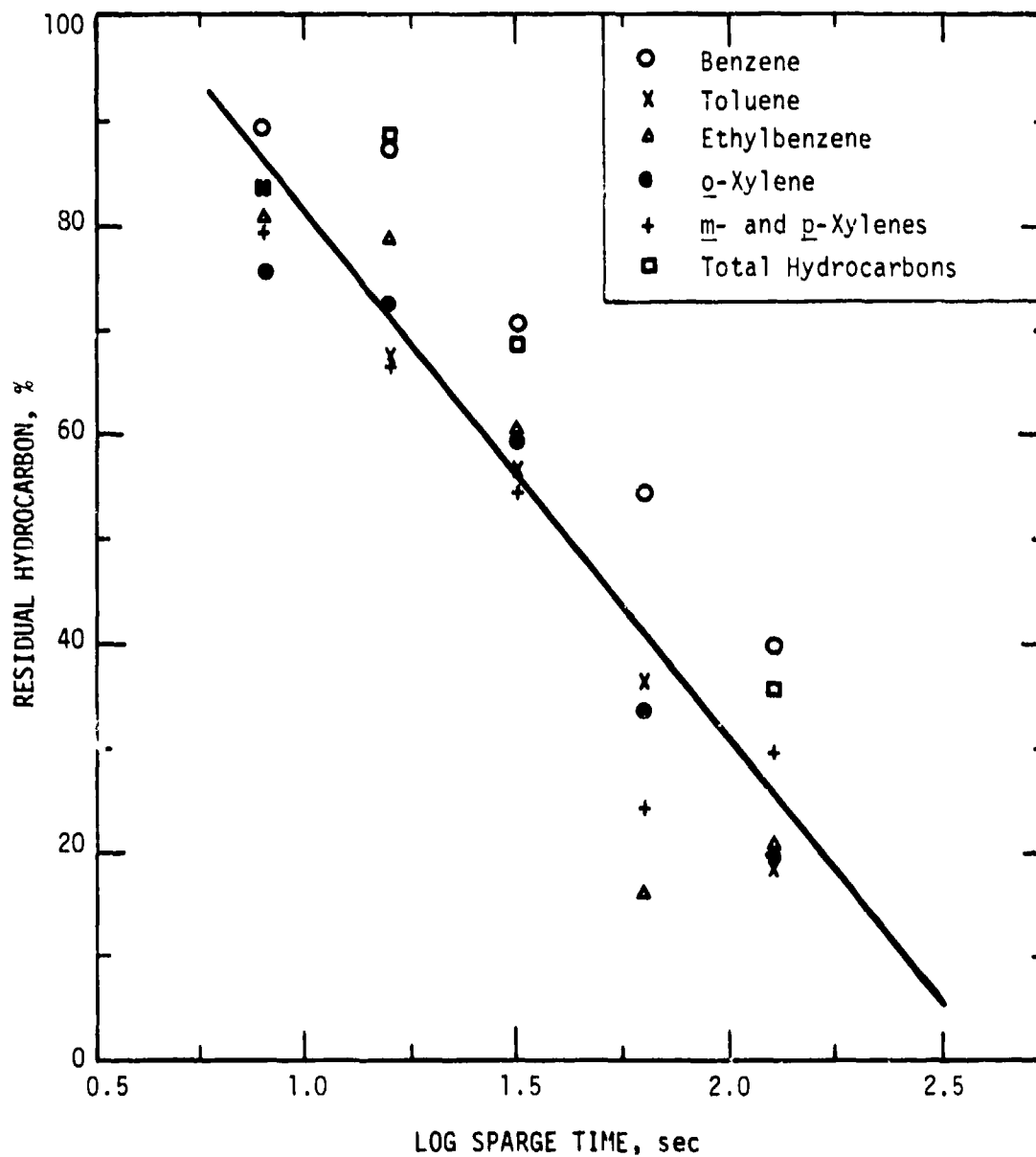


FIGURE 5. EFFECT OF SPARGE TIME ON WSF HYDROCARBONS

The data demonstrate how rapidly hydrocarbons can be removed from the jet fuel WSF (thereby emphasizing the desirability of operating in closed systems during extended bioassay tests). The semi-log plot in Figure 5 indicates a good relationship between hydrocarbon loss and sparge time. It is interesting to note that this curve is applicable both to the relatively low-boiling benzene (80°C) and the much higher boiling xylenes (145°C). This similarity in loss rates indicates that the vapor phase composition of the multicomponent azeotrope is the dominant factor in hydrocarbon removal rather than their relative volatilities at room temperature. Unfortunately, this "levelling" phenomenon effectively eliminates any possibility of relating toxicity changes to individual hydrocarbon behavior in these sparged samples.

#### Comparison of JP-4 Samples from Different Sources

An essential preliminary question that had to be answered in our investigation of the toxicity of JP-4 was whether samples of the jet fuel from different sources were sufficiently similar to permit generalizations on the basis of a single sample. In order to answer this question we requested and received the following samples from five different sources (Table 12). Analytical data provided by the suppliers are given in Appendix A.

TABLE 12  
JP-4 COMPARISON SAMPLES

Monsanto	GEC-1A-792033
Arco, Watson Refinery	JP-4-42-81, Tank R40
Friendswood Refining Corp.	Batch 66, Tank 651
Exxon Co., Baton Rouge Refinery	81-894
HRI-Geokinetics	MS 0001-792086
	(shale-derived)

The samples were compared for similarities and differences by gas chromatographic analysis of the neat fuels and their water soluble extracts (WSF), discussed below, and by their toxicity (i.e., hatching inhibition) toward Artemia salina eggs.

#### Analysis of Neat JP-4 Samples.

The neat JP-4 samples were analyzed chromatographically by direct injection of 0.15  $\mu$ L into the same column (20-foot S-2100 (10 %) on 80/100 Supelcoport) and under conditions used for previous WSF analysis (Cooper et al., 1981). The chromatograms are compared in Figure 6. A quantitative GC comparison (Table 13) of two JP-4 components which contribute a major fraction of the WSF hydrocarbons, namely benzene and toluene, was made after calibrating the GC with standards prepared in one of the jet fuels. Table 13 also contains approximate data for additional higher boiling  $C_2H_5$ -aromatics. The analyses for the latter were less accurate because these peaks are submerged in the complex mixture of peaks comprising the bulk of the JP-4 hydrocarbons. Based on the chromatograms and tabulations, the following observations were made:

1. Qualitatively the jet fuel chromatograms varied widely in their overall hydrocarbon distribution. For example, in the Friendswood sample (Figure 6 E) the peaks were bunched much more toward the first half of the chromatogram than in any other fuel. Also there were substantial differences in the presence or absence and position of the unresolved hydrocarbon "humps." Compare for example the cases of Friendswood (Figure 6 E) with virtually no hump and Exxon (Figure 6 B) with an extremely large hump.
2. Families or groups of peaks were common to all the samples but their relative amounts differed widely; compare the four-peak cluster containing the benzene peak in the different samples.
3. There were wide variations in the aromatic hydrocarbons that produced the major WSF components. Benzene and toluene concentrations, for example, covered five-fold and three-fold ranges, respectively, excluding the shale-derived JP-4. The latter was unique in containing very little aromatics in general and very low (0.05 % w/v) benzene in particular. This, of course, explains the very low WSF aromatics and low overall water solubility of the shale JP-4. The varying levels of the

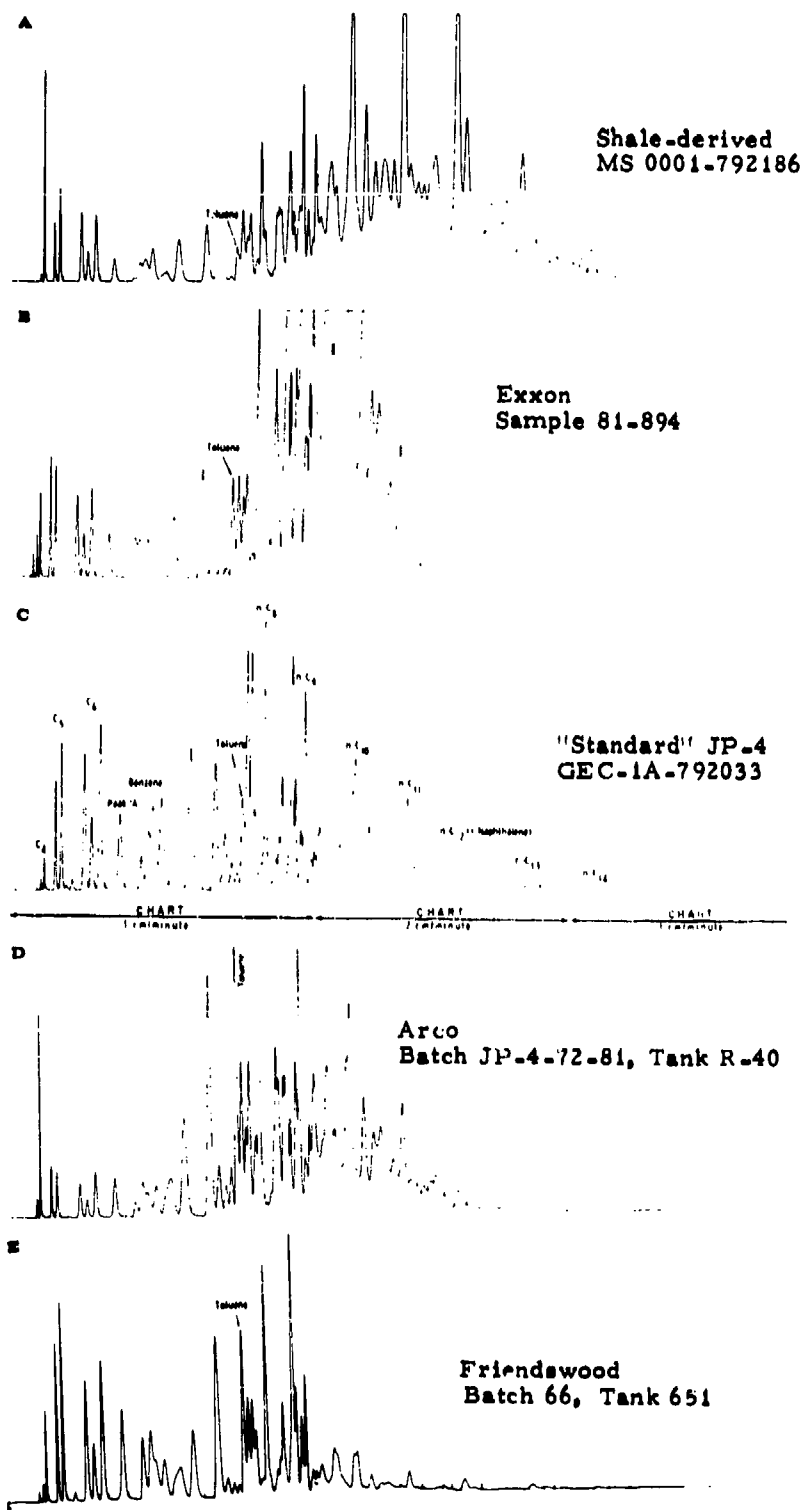


FIGURE 6. COMPARISON CHROMATOGRAMS OF NEAT JP-4 SAMPLES FROM DIFFERENT SOURCES

aromatic hydrocarbons in the neat fuels is the reason for the differences observed among the WSF's. Benzene is a good example. The highest concentration by far, 0.8 %, was found in the Friendswood fuel which in turn produced the high level of 32 ppm in its WSF. Benzene, and to a lesser extent toluene, was of particular interest because of its relatively high water solubility ( $\sim 1700$  ppm at  $20^{\circ}\text{C}$  in distilled water) and the general consensus that aromatic hydrocarbons are the toxic components of petroleum fractions.

TABLE 13  
COMPARISON OF SELECTED HYDROCARBON LEVELS  
IN DIFFERENT JP-4 SAMPLES

JP-4	Benzene $\% \text{ w/v}^a$	Toluene $\% \text{ w/v}^a$	Ethyl- benzene+ m- and p- Xylene	
			o-Xylene $\% \text{ w/v}^b$	$\% \text{ w/v}^b$
GEC-1A-792033 (Monsanto)	$0.36 \pm 0.003$	$1.18 \pm 0.01$	7	2
JP-4-42-81 (Arco)	$0.17 \pm 0.001$	$3.38 \pm 0.03$	11	3
Batch 66, Tank 651 (Friendswood)	$0.81 \pm 0.003$	$2.48 \pm 0.004$	19	5
81-894 (Exxon)	$0.34 \pm 0.004$	$1.44 \pm 0.015$	13	3
MS0001-792086 (shale-derived)	$0.05 \pm 0.001$	$0.50 \pm 0.001$	4	2

<sup>a</sup>  $\pm 1$  standard deviation

<sup>b</sup> Approximate values

- The n-alkane distribution, the predominant aliphatic hydrocarbon, was quite different in the various JP-4's. For example, although n-octane was generally the major alkane, the pattern of

decrease of the higher members from  $n\text{-C}_9$  to  $n\text{-C}_{15}$  varied from the abrupt drop-off in Arco and especially in Friendswood to the gradual decrease in the GEC-1A-792033 sample. The Exxon sample shows little evidence of significant amounts of  $n$ -alkanes above  $C_{11}$ .

5. While the Arco sample clearly shows the extra  $n\text{-C}_4$  known to be added for vapor pressure control, the shale sample appears to contain at least as much.

#### Analysis of Water Soluble Extracts (WSF).

WSF's were prepared from each JP-4 by our standard procedure (gentle magnetic stirring for a minimum 24 hours at  $20^\circ\text{C}$  of a lower phase consisting of 1.6% "Instant Ocean" in ASTM Type I water overlaid by the jet fuel). Samples were analyzed by pentane extraction/GC using  $n$ -hexadecane as the internal reference standard. For the analysis of  $n$ -pentane (and  $n$ -butane) the WSF's were extracted with  $n$ -heptane instead of  $n$ -pentane. Typical chromatograms at high and low attenuation, to show the major and minor components respectively, are displayed in Figures 7 and 8. A quantitative comparison of the major components is given in Table 14. Somewhat less accurate estimates (because of very small overlapping peaks) for three minor but potentially toxic hydrocarbons, naphthalene (a fused ring aromatic), tetralin, and decalin (cycloparaffins) are given in Table 15 for the JP-4 samples.

The following observations are relevant:

1. As expected, clear substantial differences in hydrocarbon composition of the WSF of the various JP-4 fuels were evident. These differences were present both in the major and minor components. They can be seen by visual comparison of the chromatograms and in the detailed analysis of the selected hydrocarbon peaks. Substantial differences were also visible in the shape and position of the "hump" of unresolved components at the lower attenuation (Figure 7).
2. There were substantial variations, covering the range of 2- to 3-fold (excluding the shale-derived material), in the total amounts of hydrocarbons dissolved in the aqueous phase. The higher

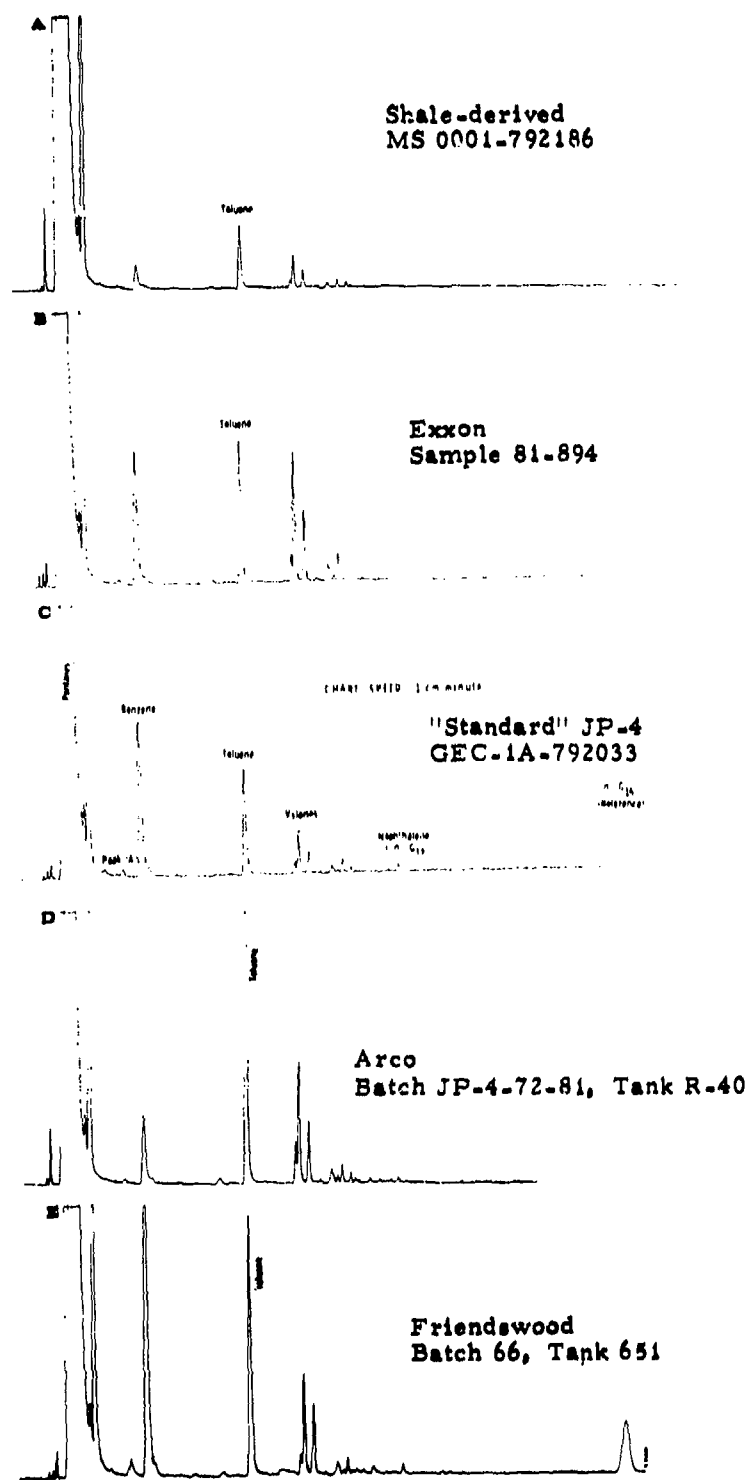


FIGURE 7. COMPARISON CHROMATOGRAMS OF WATER SOLUBLE EXTRACTS OF JP-4  
SAMPLES FROM DIFFERENT SOURCES (Run at high attenuation  
[210] to show major peaks.)





TABLE 14  
COMPARISON OF MAJOR HYDROCARBON COMPONENTS IN WSF'S FROM DIFFERENT JP-4 SAMPLES  
(Prepared in 1.6 % "Instant Ocean" at 20°C)

	Petroleum-derived JP-4				Shale-derived JP-4
	Monsanto <sup>a</sup> ppm w/v	Arco <sup>a</sup> ppm w/v	Friendswood <sup>a</sup> ppm w/v	Exxon <sup>a</sup> ppm w/v	HRI- Geokinetics <sup>a</sup> ppm w/v
Benzene ("B")	11.3 ± 0.5 <sup>b</sup>	6.4 ± 0.3	29.3 ± 1.5	12.2 ± 0.7	2.04 ± 0.05
Toluene	7.8 ± 0.8	25.8 ± 0.9	18.9 ± 0.7	11.2 ± 0.4	5.0 ± 0.3
Ethylbenzene	0.62 ± 0.06	2.4 ± 0.11	1.19 ± 0.08	1.41 ± 0.06	0.38 ± 0.01
m + p-Xylenes	2.1 ± 0.16	6.6 ± 0.2	5.4 ± 0.2	7.1 ± 0.2	1.79 ± 0.04
o-Xylene	1.03 ± 0.07	3.1 ± 0.2	4.3 ± 0.2	3.5 ± 0.1	0.82 ± 0.03
Peak "A"	0.39 ± 0.03	0.19 ± 0.01	0.37 ± 0.05	0.22 ± 0.01	0.12 ± 0.01
n-Pentane	0.90 ± 0.02	0.42 ± 0.04	1.11 ± 0.04	0.80 ± 0.03	0.79 ± 0.01
n-Butane	0.45 ± 0.02	1.94 ± 0.12	0.87 ± 0.05	0.86 ± 0.05	2.70 ± 0.14
Total Hydrocarbon	28.5 ± 1.6	51.6 ± 0.7	70.6 ± 2.4	41.8 ± 2.4	13.9 ± 0.4
Peak "A" X 100	3.5 %	3.5 %	2.9 %	1.8 %	5.7 %
Peak "B"					

<sup>a</sup> See Table 12 for sample designations

<sup>b</sup> ± 1 standard deviation

concentrations were accounted for by the larger amounts of aromatics in these WSF's. The latter in turn were correlated with higher proportions present in the corresponding neat JP-4 (see Table 13).

3. The relative amounts of the major individual aromatic hydrocarbons varied independently. For example, benzene was ~ 36 % lower in Arco and ~ 300 % higher in Friendswood than in the Monsanto sample (GEC-1A-792033), although both the former had higher overall aromatic levels than the latter. In the case of Arco, the low benzene was offset by a very high proportion of toluene (50 % of the total hydrocarbon). A comparison of the major aromatics composition by relative percent is given in Table 16. It is interesting to note the close similarity in relative composition in the Monsanto and Friendswood samples although the absolute levels were nearly three times higher in

the latter. This suggested that a similar aromatic fraction was used in these jet fuels but at ~ three times higher concentration in the Friendswood JP-4.

4. On the basis of the bioassay data, the ranking by toxicity did not appear to correlate well with the varying amounts of individual hydrocarbons. Benzene, thought to be a prime toxicant candidate in petroleum fractions, is a good example. The Exxon WSF contained twice as much as the Arco WSF, whereas their toxicities in no way reflect this 2-fold difference.
5. The variations in the amounts of the three minor peaks thought to be (at least in part) naphthalene, tetralin, and trans-decalin were quite large (Table 15). Again these variations did not parallel the WSF toxicities suggesting that none of these hydrocarbons played a major role in the fuel toxicity.
6. The data for the low boiling alkanes, n-pentane and n-butane, not previously reported because their chromatogram peaks were masked by our pentane extracting solvent, indicated that their levels were in the same 0.5—2.0 % range as adjacent aliphatic peaks such as the presumptive alkane peak "A" used by us to monitor for emulsion contamination in our WSF's. Again the levels within this range were quite variable (pentane over a 3-fold range, butane over a 6-fold range) among the different JP-4 samples.
7. The substantially lower water solubility of the shale JP-4 was of particular interest. At 13 ppm it was less than half the solubility of any other JP-4. This, of course, was a direct consequence of the greatly reduced levels of aromatics, the most water soluble components of the jet fuel.
8. The value of the ratio of peak "A" (an unidentified alkane emerging just ahead of benzene) to benzene has proven to be very consistent in the large proportion of our work performed with the single JP-4 sample, GEC-1A-792033. As such it has been a useful monitor for the presence of fuel emulsion in the water phase since the ratio in the neat fuel that would be a part

of any emulsion would be several hundred-fold greater ( $\sim 300\%$ ) (see for example the relative peak sizes for peak "A" and benzene in the neat fuel chromatogram in Figure 6 C). In view of this consistency, the widely different values for the ratio in different JP-4 WSF's would appear to be a useful aid in characterizing the fuels.

On the basis of the above physico-chemical examination of these five JP-4 samples, it was concluded that there were substantial differences in fuels from different sources (and presumably from the same source as feedstocks varied over a period of months or years). The key element, of course, was whether these differences were reflected in toxicity differences.

TABLE 15  
COMPARISON OF THREE MINOR HYDROCARBON COMPONENTS  
IN WSF'S FROM DIFFERENT JP-4'S

	WSF Source		
	Monsanto ppm w/v	Arco ppm w/v	Friendswood ppm w/v
Naphthalene (+ n-C <sub>12</sub> ) <sup>a</sup>	0.42	0.40	0.59
Tetralin	0.05	0.15	0.05
Decalin ( <u>trans</u> )	0.04	0.03	$\sim 0.01$

<sup>a</sup> Naphthalene and n-C<sub>12</sub> have same retention time. WSF peak is presumed to be largely naphthalene in view of the much higher water solubility of the latter.

TABLE 16  
RELATIVE COMPOSITION OF THE MAJOR AROMATICS GROUP  
IN DIFFERENT JP-4 SAMPLES

	Petroleum-derived JP-4				Shale-derived JP-4
	Monsanto %	Arco %	Friendswood %	Exxon %	HRI- Geokinetics %
Benzene	49	14	50	34	20
Toluene	34	58	32	32	50
Ethylbenzene	3	5	2	4	4
m- + p-Xylenes	9	15	9	20	18
o-Xylene	5	7	7	10	8

Preparation and Analysis of WSF's from Hydrocarbon Mixtures of Known Composition

A number of WSF's were prepared from "synthetic" hydrocarbon mixtures of known composition. Two of the mixtures, a mix of the major fuel WSF aromatics (benzene, toluene, ethylbenzene, xylenes) and a naphthalene/decalin/tetralin mix, were designed to simulate JP-4 WSF's in tests for synergistic effects. Several other mixed aromatic/alkane compositions were prepared and used to test for additive or synergistic toxicity in hydrocarbon mixtures. During the preparation of these latter WSF's, the feasibility of predicting WSF composition from the known hydrocarbon mix composition was investigated. The WSF's were prepared by our usual 2-phase contacting procedure.

Benzene/Toluene/Ethylbenzene/Xylenes Mixture.

This mixture was prepared to simulate the composition of the JP-4 (GEC-1A-792033) WSF in these aromatic components. Separate WSF's were prepared and analyzed for each pure hydrocarbon in the mixture. Based on the analyses (Table 9), the calculated amounts of each WSF, necessary to produce the desired final WSF composition, were combined. Analysis of the mixed "WSF" (Table 17) showed excellent agreement with the target JP-4 WSF.

TABLE 17  
ANALYSIS OF "SIMULATED" JP-4 WSF CONTAINING BENZENE,  
TOLUENE, ETHYLBENZENE, AND XYLENES

	Desired Composition ppm w/v	Analyzed Composition ppm w/v
Benzene	33.0 <sup>a</sup>	31.8
Toluene	21.9	20.4
Ethylbenzene	1.74	1.65
<u>m</u> -Xylene {	6.04 <sup>b</sup>	5.31
<u>p</u> -Xylene }		
<u>o</u> -Xylene	2.97	2.49

<sup>a</sup> 3 X levels in JP-4 GEC-1A-792033 since bioassay test dilutions were started at this higher level

<sup>b</sup> m- and p-xylenes were assumed to be present in equal amounts since their relative contributions to their common GC peak were unknown

#### Naphthalene/Decalin/Tetralin Mixture.

In order to test decalin, tetralin, and naphthalene at levels comparable to those in JP-4 (GEC-1A-792033) WSF, it was necessary to locate them in the WSF chromatogram by the addition of authentic samples (Figure 10).

Assuming identity with the coincident WSF peaks, the amounts of trans-decalin and tetralin in the WSF were very low, ~0.04 ppm. The peak at the naphthalene position was substantially larger, ~0.4 ppm. n-Dodecane has the same retention time. However, it was assumed that the peak was largely due to naphthalene based on 1) the smallness of the n-undecane peak and the assumed similar fuel/water partitioning behavior of n-dodecane and n-undecane and 2) the much greater water solubility of naphthalene relative to n-dodecane. Although accurate analysis of these hydrocarbons in the WSF was impossible because of the crowding and overlapping of many low level components, the approximate figures quoted above were considered acceptable targets for the desired "synthetic" naphthalene/decalin/tetralin mix.

As with the above benzene/toluene/xylenes mix, separate WSF's were prepared from each component. However, because the solid naphthalene posed potential handling and mixing problems, a modified procedure was

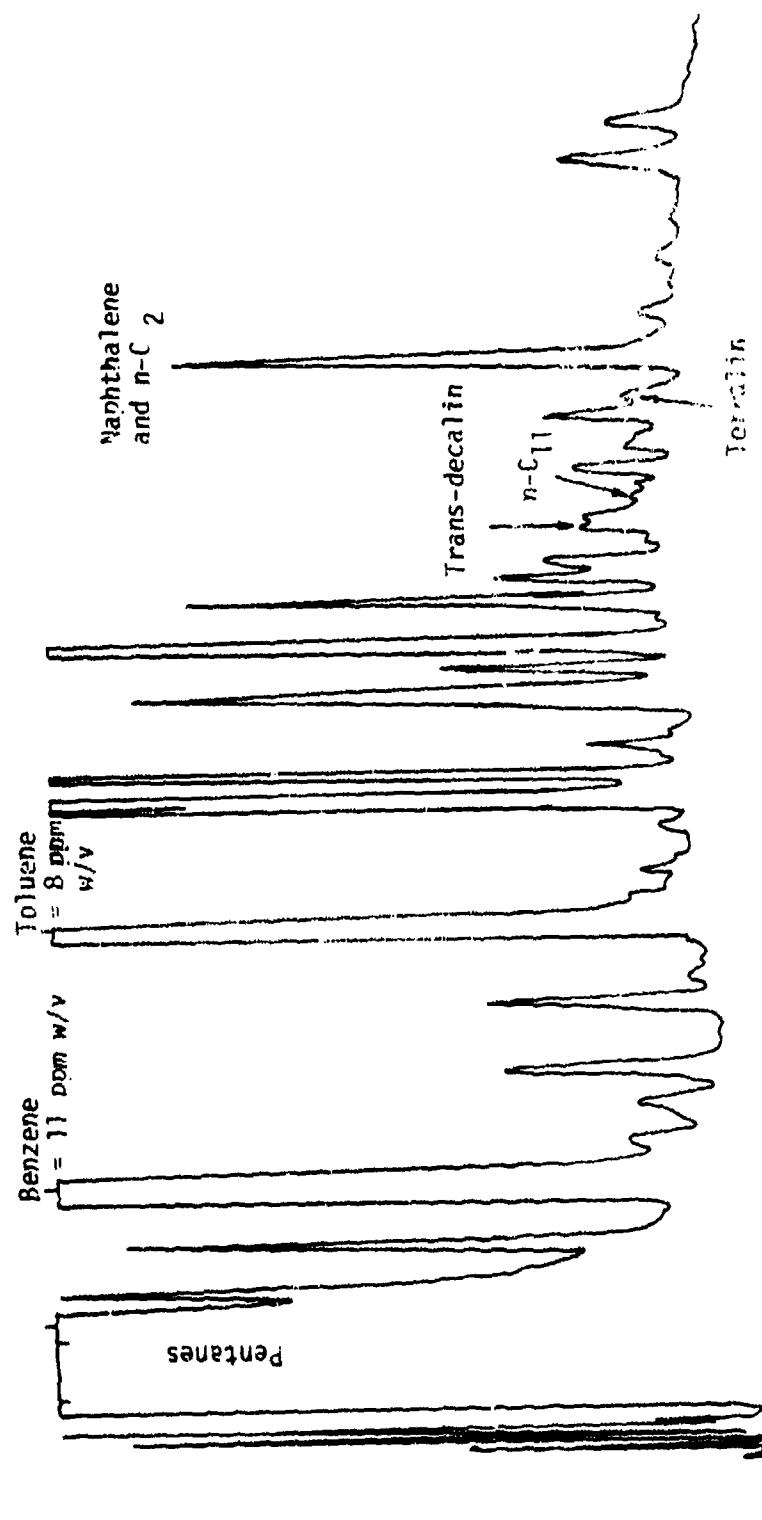


FIGURE 9. CHROMATOGRAM OF WATER SOLUBLE EXTRACT FROM JP-4 (GEC-1A-792033) SHOWING LOCATION OF TRANS-DECALIN, TETRALIN, NAPHTHALENE/n-C<sub>12</sub> AND n-C<sub>11</sub> PEAKS

developed using a solution of the hydrocarbon in an appropriate solvent. Solvents tested included decalin and tetralin, preferred because they were already components of the final mixture, and benzene. Naphthalene solubility in decalin was much too low. Tests with naphthalene/tetralin and naphthalene/benzene solutions gave the following WSF compositions (Table 18).

TABLE 18  
SOLUBILITY OF NAPHTHALENE MIXTURES IN WATER  
(1.6 % "INSTANT OCEAN") AT 20°C

	WSF Composition		
	Naphthalene ppm w/v	Tetralin ppm w/v	Benzene ppm w/v
Naphthalene/tetralin (1:3.2 pts w/v)	22.3	29.2	-
Naphthalene/benzene (1:2.2 pts w/v)	22.1	-	1074

The desired ratio of 10:1 naphthalene/tetralin in the final mixed WSF was not attainable with the naphthalene/tetralin organic phase. Naphthalene/benzene, however, gave adequate naphthalene levels at dilutions where the accompanying benzene would not interfere with the desired bioassay. Combination of individual WSF's in appropriate proportions gave a satisfactory simulation of the JP-4 WSF (Table 19).

#### Aromatic/Alkane Test Mixtures.

WSF's of model mixtures of aromatic and aliphatic components were of interest for the investigation of additivity and synergism in the toxicity of jet fuels. WSF systems were prepared from mixtures of known amounts of benzene, toluene, *n*-pentane, and *n*-heptane. The two alkanes, at the lower end of the JP-4 range, were preferred over the higher boiling alkanes in the median JP-4 range because the very low water solubilities of the latter made them difficult or impossible to work with, especially in the determination of LC 50 values. The WSF's were prepared in the usual

manner. It has been stated (McAuliffe, 1976) that individual water solubilities of hydrocarbons in a mixture are determined by their mole fraction in the mixture and their true single hydrocarbon solubility.

TABLE 19  
ANALYSIS OF "SIMULATED" JP-4 WSF CONTAINING  
NAPHTHALENE/DECALIN/TETRALIN (AND BENZENE)

	Desired Composition <sup>a</sup> ppm w/v	Analyzed Composition ppm w/v
Decalin		
(i) <u>trans</u> -isomer	0.12	0.083
(ii) <u>cis</u> -isomer	0.205 <sup>b</sup>	0.215
Tetralin	0.12	0.115
Naphthalene/benzene <sup>c</sup>		
(i) Naphthalene	1.20	1.35
(ii) Benzene	93 <sup>d</sup>	85

<sup>a</sup> Approximately 3 times levels in JP-4 (GEC-1A-792033) WSF

<sup>b</sup> Estimated level in volume of decalin WSF calculated to contain 0.12 ppm trans-isomer

<sup>c</sup> WSF prepared from a 1:2.3 pts w/v naphthalene/benzene solution

<sup>d</sup> Estimated level in volume of naphthalene/benzene WSF calculated to contain 1.2 ppm naphthalene

It was of interest to compare WSF composition predicted on this basis with the analyzed composition (Table 20). Agreement with prediction was excellent for the alkanes but very poor for the aromatic hydrocarbons. Benzene was nearly twice as soluble and toluene ~ 1.7 times as predicted, presumably as a result of their greater polarity vis-a-vis the alkanes.



TABLE 20

MIXED AROMATIC/ALIPHATIC MODEL WSF'S<sup>a</sup> - COMPARISON  
OF PREDICTED AND ANALYZED COMPOSITION

Mix A	Organic Phase		WSF			
	Parts by Volume	Mole Fraction	Predicted <sup>b</sup>	Analyzed		
			ppm w/v	Mix I ppm w/v	Mix II ppm w/v	Mix III ppm w/v
Benzene	3	0.068	68	142	146	145
Toluene	3	0.056	22	37	37	37
<u>n</u> -Pentane	16	0.277	1.35	1.34	1.6	1.4
<u>n</u> -Heptane	44	0.599	9.2	10.0	9.9	9.7
<u>Mix B</u>						
Benzene	3	0.031	31		58	
Toluene	6	0.047	18		30	
<u>n</u> -Pentane	25	0.181	6.0		6.5	
<u>n</u> -Heptane	130	0.740	1.7		1.9	

<sup>a</sup> In 1.6 % "Instant Ocean"

<sup>b</sup> Based on mole fraction X individual hydrocarbon solubilities:

benzene	1000 ppm
toluene	388 ppm
<u>n</u> -pentane	33.3 ppm
<u>n</u> -heptane	2.25 ppm

## BIOASSAYS

### INTRODUCTION

Toxicity bioassays were performed on three different classes of hydrocarbons, namely 1) JP-4 jet fuels, 2) individual hydrocarbon components of these jet fuels, and 3) synthetic hydrocarbon mixtures designed to simulate some portion of the JP-4 constituents. The jet fuels were bioassayed for comparison purposes while the individual hydrocarbons and mixtures were analyzed as part of the investigation into the nature and source of the toxic components in JP-4.

Bioassays were based on the inhibition of hatching of the eggs (cysts) of the brine shrimp, Artemia salina during a 48-hour exposure to dilutions of a water soluble extract (WSF) of the hydrocarbon(s). The Bioassay Development section describes some of the factors associated with satisfactory performance of this assay. The following is a generalized description of the experimental procedure that was used with all the different kinds of samples.

Artemia eggs (100 mg) were prepared for the bioassay by prehydration in aerated distilled water (50 mL) for 1 hour. The eggs were collected on a fine mesh nylon screen and rinsed into a beaker with bleach solution (200 mL bleach + 100 mL H<sub>2</sub>O). After stirring for 3-5 minutes the dechoriation was stopped with a water rinse followed by copious washing in running water for 1 hour. The eggs were transferred to a 1.6% solution of "Instant Ocean" (Aquarium Systems, Mentor, Ohio) in ASTM Type I water. One hundred eggs per tube were counted into 50 mL ground glass stoppered centrifuge tubes. The appropriate amount of WSF (JP-4 or other hydrocarbon) to give the desired dilution was added to fill the tube completely, leaving no air space when the ground glass stopper was inserted. The eggs were incubated at 26°C for 48 hours, turning the tubes after 24 hours. The hatch count was taken at 48 hours. Alternatively, the 48-hour sample was preserved by the addition of formaldehyde for later counting.

The experimental data were used to calculate LC 50 values by the moving average method. The computation was initially performed using a program developed for the Sharp EL 5100 calculator. Later a computer

program in Basic (Sinclair Extended) was written to facilitate the calculation. Details of the procedure and computer program are given in Appendix II.

#### COMPARISON OF JP-4 TOXICITIES

In our investigation of the toxic effects of the jet fuel JP-4 an important question that required an early answer was whether JP-4 samples from different producers were sufficiently similar to allow one to generalize on the basis of the single sample that we originally received. To answer this question we requested and received a total of five different samples, listed in Table 12. Bioassay data from the WSF's prepared from these samples are summarized in Tables 21-25. LC 50 values were computed and are given in Table 26.

The data indicated that there were substantial differences in toxicity between the different JP-4 samples. While three of the samples, Monsanto, Arco, and Exxon, were essentially similar, Friendswood JP-4 was almost twice as toxic. The latter was also more than three times as toxic as the shale-derived JP-4. The latter was by far the least toxic, a surprising observation in view of the general opinion prior to these tests that the shale-derived fuel was likely to be more toxic than the petroleum samples. It is important to note that these comparisons were based on LC 50 values that were expressed in terms of percent WSF. This of course is a practical and meaningful basis for assessing the environmental impact of different fuel spills since it compares toxicities on a volume basis. However, the results can be quite misleading, especially where a complex mixture of components is involved with varying degrees of individual toxicity. This can be seen when toxicity is reported in terms of parts per million total hydrocarbon (Table 26). On this basis the shale-derived JP-4 was the most toxic, albeit by a smaller margin. This changed ranking seemed to reflect the different water solubilities of the JP-4's and suggested a direct relationship between toxicity expressed as percent WSF and overall solubility rather than with the presence of one (or at most a few) specifically toxic components. This point is significant in view of the results with individual hydrocarbons and hydrocarbon mixtures reported in a later section.

TABLE 21

JP-4 (GEC-1A-792033)(MONSANTO) - Artemia salina

## HATCHABILITY BIOASSAYS

Conditions: eggs, 100/dilution; incubation temperature 26° C

Series I			Series II			Series III		
WSF Concen- tration % v	Hatch No.	Relative Hatch- ability %	Hatch No.	Relative Hatch- ability %	WSF Concen- tration % v	Hatch No.	Relative Hatch- ability %	
0	42 28	100	55 45	100	0	68 63 52	100	
10	41	117	47	94	15	43	70	
20	28	80	32	64	20	42	69	
30	14	40	34	68	25	21	34	
40	6	17	8	16	30	16	26	
50	3	8.6	8	16	35	3	5	
60	0	0	0	0	40	0	0	
70	0	0	0	0	45	7	11	

TABLE 22

JP-4 (ARCO JP4-42-8) - Artemia salina

## HATCHABILITY BIOASSAYS

Conditions: eggs 100/dilution; incubation temperature 26° C

Series I			Series II			Series III		
WSF Concen- tration % v	Hatch No.	Relative Hatch- ability %	WSF Concen- tration % v	Hatch No.	Relative Hatch- ability %	Hatch No.	Relative Hatch- ability %	
0	42 28	100	0	55 68 55	100	66 68 67	100	
10	23	66	5	-	-	62	93	
20	17	49	10	57	96	63	94	
30	24	69	15	53	89	47	70	
40	1	3	20	35	59	49	73	
50	0	0	25	9	15	37	55	
60	1	3	30	2	3.4	7	10	
70	0	0	35	1	1.7	1	1.5	
			40	0	0	-	-	

TABLE 23

JP-4 (FRIENDSWOOD, BATCH 66 TANK 651) - Artemia salina

## HATCHABILITY BIOASSAYS

Conditions: eggs, 100/dilution; incubation temperature 26° C

WSF Concen- tration % v	Series I		WSF Concen- tration % v	Series II	
	Hatch No.	Relative Hatch- ability %		Hatch No.	Relative Hatch- ability %
0	42 } 28 }	100	0	52 } 46 } 65 }	100
10	24	69	4	46	85
20	8	23	8	37	68
30	5	14	12	30	55
40	0	0	16	27	50
50	1	3	20	16	29
60	0	0	24	5	9
70	2	6	28	0	0

TABLE 24

JP-4 (EXXON, 81-894) - Artemia salina

## HATCHABILITY BIOASSAYS

Conditions: eggs, 100/dilution; incubation temperature 26° C

WSF Concen- tration % v	Series I		WSF Concen- tration % v	Series II	
	Hatch No.	Relative Hatch- ability %		Hatch No.	Relative Hatch- ability %
0	55 } 45 }	100	0	58 } 56 } 54 }	100
10	30	60	10	55	98
20	28	56	20	33	59
30	22	44	30	15	27
40	4	8	40	4	7
50	3	6	50	0	0
60	0	0	60	0	0
70	0	0	70	0	0

TABLE 25

SHALE JP-4 (MS 0001-792086) - Artemia salina  
HATCHABILITY BIOASSAYS

Conditions: eggs, 100/dilution; incubation temperature 26° C

WSF Concen- tration % v	Series I		WSF Concen- tration % v	Series II	
	Hatch No.	Relative Hatch- ability %		Hatch No.	Relative Hatch- ability %
0	55 } 45 }	100	0	55 } 61 } 55 }	100
10	53	106	35	46	81
20	51	102	40	32	56
30	33	66	45	29	51
40	36	72	50	18	32
50	20	40	55	12	21
60	23	46	60	5	9
70	10	20	65	6	10.5

TABLE 26

## COMPARISON OF LC 50 VALUES FOR DIFFERENT JP-4 SAMPLES

Fuel <sup>a</sup>	LC 50			
	Individual Bioassays, % WSF		Mean Values	
	LC 50	95% Conf. Limits	% WSF $\pm$ 1 SD	ppm Total <sup>b</sup> HC $\pm$ 1 SD
Monsanto	30.9	1.4	27.3 $\pm$ 4.2	7.7 $\pm$ 1.2
	28.4	2.3		
	22.7	1.7		
Arco	25.4	3.1	23.0 $\pm$ 2.4	11.9 $\pm$ 1.2
	20.7	0.5		
	22.9	0.65		
Friendswood	14.0	0.7	13.8 $\pm$ 0.3	9.7 $\pm$ 0.2
	13.6	3.6		
Exxon	21.9	3.5	22.8 $\pm$ 1.3	9.5 $\pm$ 0.5
	23.7	1.6		
Shale	49.5	2.5	46.3 $\pm$ 4.3	6.4 $\pm$ 0.6
	43.2	1.7		

<sup>a</sup> See Table 12 for sample designations

<sup>b</sup> Based on mean total WSF hydrocarbon values in Table 14

## EFFECT OF SPARGING ON WSF TOXICITY

When the toxicity of individual aromatic hydrocarbons comprising the major components of the JP-4 WSF's proved to be surprisingly low (see next section) it was hypothesized that a major portion of the toxicity might be due to a) non-hydrocarbon and therefore more polar components, e.g. additives, of the fuel or b) less volatile higher molecular weight hydrocarbons. In either case it seemed likely that the removal of the more volatile hydrocarbons from the WSF by sparging with nitrogen or air would allow such materials to accumulate in the WSF. Thus the toxicity of the residual material would be expected to increase or, more correctly, it would decrease at a slower rate than the hydrocarbon loss. Physical details and analyses of the two series of sparging experiments were described earlier in this report (see page 27 and Table 11). Bioassay data on the sparged WSF's are tabulated below (Table 27). The effects of sparging and dilution

TABLE 27

### EFFECT OF SPARGING ON JP-4 (GEC-1A-792033) WSF TOXICITY - Artemia salina HATCHABILITY BIOASSAYS

Conditions: eggs, 100/Sparge Sample, incubation temperature 26°C

Sparge Time sec	Series I		Series II		Series III		
	Hatch No.	Relative Hatch- ability %	Hatch No.	Relative Hatch- ability %	Sparge Time sec	Hatch No.	Relative Hatch- ability %
0	70	100		100	0 <sup>a</sup>	74	100
	73						
0	2	3	3	4	0	0	0
120	64	90	61	85	2	0	0
240	64	90	64	90	4	0	0
480	71	99	67	94	8	0	0
960	68	95	67	94	16	0	0
1920	78	109	72	101	32	6	8
					64	57	77
					128	69	93

<sup>a</sup> Control - distilled water (no WSF)

are compared in Figure 10. The data showed that the decrease in toxicity paralleled, and in fact appeared to exceed, the loss from the simple reduction by dilution in hydrocarbon concentration, in contrast to the lag expected if the more toxic components were accumulating in the sparged WSF. Thus there was no evidence from these experiments for the presence of highly toxic, low volatility components in the WSF. To the contrary, the essentially complete loss of toxicity after sparging for only 2-3 minutes indicated the ready volatility of the toxic materials and suggested that they were unlikely to be polar non-hydrocarbon compounds (additives).

#### TOXICITY OF INDIVIDUAL HYDROCARBON COMPONENTS OF JP-4 WSF

As noted in last year's report (1981), JP-4 WSF's consist predominantly (up to 90 %) of the three simplest aromatic hydrocarbons: benzene, toluene, and the xylenes. Since the toxicity of petroleum fractions is persistently attributed to the presence of these aromatic compounds, especially benzene (Rice et al., 1976; Moore and Dwyer, 1974; Blumer, 1971) it was of prime importance to investigate their toxicity and the toxicity of these mixtures (for the latter see next section) as measured by our hatchability bioassay. The resulting raw data for benzene and toluene and their computed LC 50 values are tabulated in Tables 28, 29, and 33. The toxicity of these major WSF components proved to be surprisingly low at 66 ppm and 40 ppm, respectively, when compared with the JP-4 toxicities (Table 26). Consequently, their contribution to JP-4 toxicity was relatively slight. This was evident from their low levels, 3.1 ppm for benzene and 2.1 ppm for toluene, in the WSF LC 50 dilution for a typical medium-toxicity jet fuel (GEC-1A-792033). At these levels their toxic contribution obviously could not be any greater than ~ 5 %, assuming no synergistic effects in the mixed hydrocarbon system. Synergism must be considered as one possible explanation for the high JP-4 toxicity and this possibility was tested with the hydrocarbon mixtures. Other possible explanations included the presence of much more toxic compounds among the minor JP-4 components.

Before investigating the possible presence of toxic water-soluble fuel additives (the latter in any case appeared to be unlikely toxic candidates in view of the results of our sparging tests), some of the



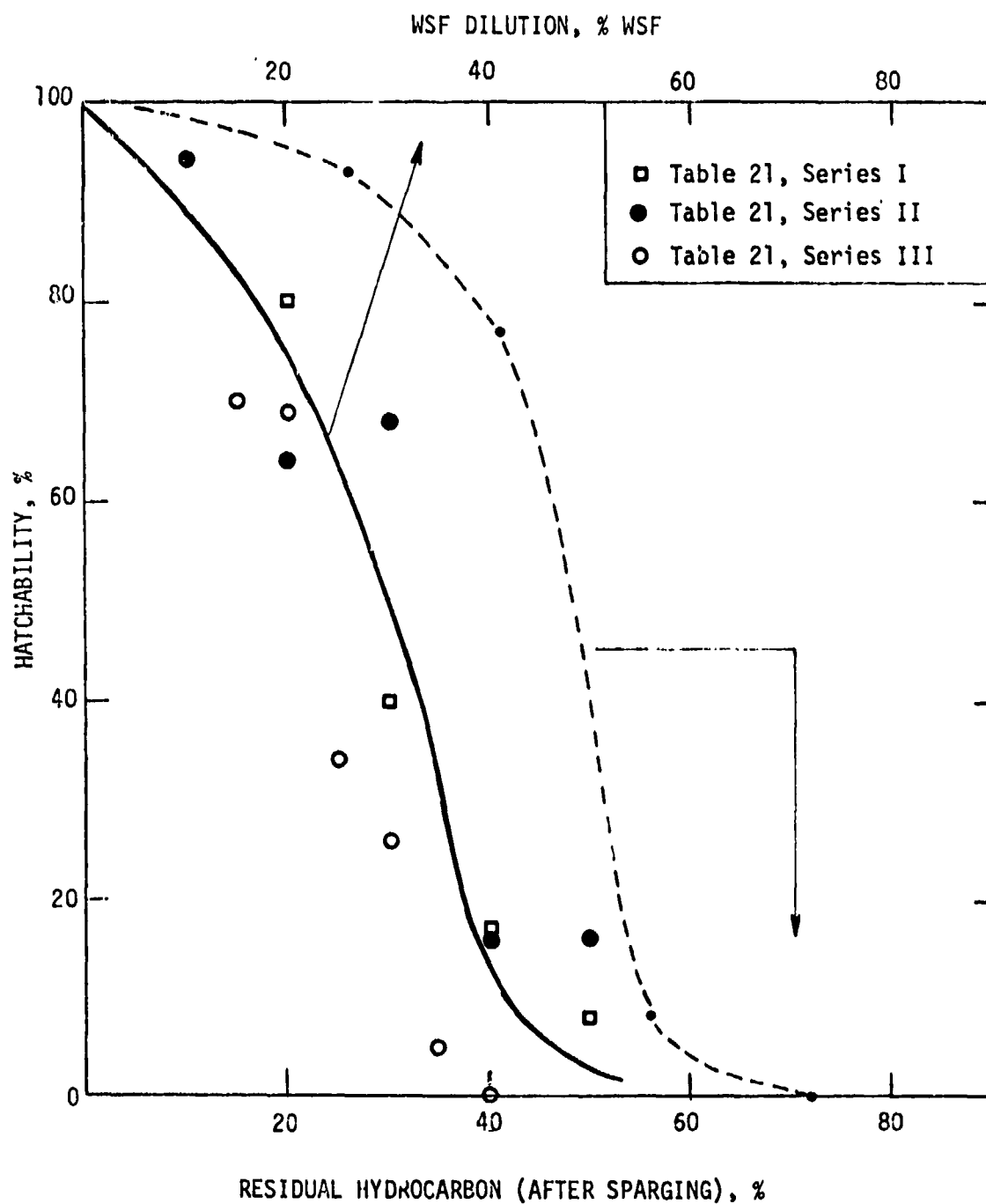


FIGURE 10. COMPARISON OF SPARGING AND DILUTION OF JP-4 WSF ON Artemia HATCHABILITY

TABLE 28

BENZENE WSF - Artemia salina HATCHABILITY BIOASSAY

Conditions: eggs, 100/dilution; incubation temperature 26°C

Log (Benzene Conc., ppm)	Relative Hatchability, %		Log (Benzene Conc., ppm)	Relative Hatchability, %	
	Series I	Series II		Series III	Series IV
0	100 <sup>a</sup>	100 <sup>a</sup>	0	100 <sup>b</sup>	100 <sup>b</sup>
1.07(1.0) <sup>c</sup>	73	77	1.51(2.5) <sup>c</sup>	88	86
1.27(1.6)	7	78	1.71(4.0)	72	64
1.47(2.5)	66	74	1.91(6.3)	28	51
1.67(4.0)	73	69	2.11(10.0)	32	35
1.87(6.3)	61	46	2.31(15.8)	0	0
2.07(10.0)	11	5			

<sup>a</sup> Control hatch numbers were 72, 74 (per 100 eggs)<sup>b</sup> Control hatch numbers were 69, 69 (per 100 eggs)<sup>c</sup> Numbers in parentheses represent % WSF by volume in the dilution

TABLE 29

TOLUENE WSF - Artemia salina HATCHABILITY BIOASSAY

Conditions: eggs, 100/dilution; incubation temperature 26°C

Series I		Series II		Series III	
Log (Toluene Conc., ppm)	Relative Hatchability %	Toluene Conc. ppm	Relative Hatchability %	Toluene Conc. ppm	Relative Hatchability %
0	100 <sup>a</sup>	0	100 <sup>b</sup>	0 <sup>d</sup>	100 <sup>c</sup>
1.07(3.6) <sup>d</sup>	76	24.9(6) <sup>d</sup>	106	44.4(10)	52
1.22(5.0)	95	33.3(8)	38	53.2(12)	23
1.37(7.1)	65	41.6(10)	72	62.1(14)	34
1.52(10.0)	76	49.9(12)	10	71.0(16)	0
1.67(14.2)	21	58.2(14)	0		
1.82(20.0)	0	66.5(16)	0		
		74.8(18)	0		

<sup>a</sup> Control hatch numbers were 65, 61 (per 100 eggs)<sup>b</sup> Control hatch numbers were 60, 62, 58 (per 100 eggs)<sup>c</sup> Control hatch numbers were 58, 56, 54 (per 100 eggs)<sup>d</sup> Numbers in parentheses represent % WSF by volume in the dilution

TABLE 30

n-PENTANE WSF - Artemia salina HATCHABILITY BIOASSAY

Conditions: eggs, 100/dilution; incubation temperature 26° C

Log (Pentane Concentration) ppm w/v	Relative Hatchability, %	
	Series I	Series II
0	100 <sup>a</sup>	100 <sup>b</sup>
0.37 (7.0) <sup>c</sup>	87	87
0.59 (11.7)	71	73
0.81 (19.4)	20.7	43
1.04 (32.4)	0	0
1.26 (54.0)	0	0
1.48 (90.0)	0	-

<sup>a</sup> Control hatch numbers were 67, 68 (per 100 eggs)<sup>b</sup> Control hatch numbers were 65, 61 (per 100 eggs)<sup>c</sup> Numbers in parentheses represent % WSF by volume in dilution

TABLE 31

n-HEPTANE WSF - Artemia salina HATCHABILITY BIOASSAY

Conditions: eggs, 100/dilution; incubation temperature 26° C

Series I		Series II	
Log (Heptane Concentration) ppm w/v	Relative Hatchability %	Heptane Concentration ppm w/v	Relative Hatchability %
0	100 <sup>a</sup>	0 <sup>b</sup>	88
-0.8 (7) <sup>c</sup>	94	0.22 (10) <sup>c</sup>	95
-0.59 (11.7)	104	0.56 (25)	91
-0.37 (19.4)	106	0.90 (40)	121
-0.14 (32.4)	83	1.24 (55)	71
0.08 (54.0)	66	1.58 (70)	4
0.30 (90.0)	28	1.92 (85)	13
		2.26 (100)	

<sup>a</sup> Control hatch numbers were 64, 57 (per 100 eggs)<sup>b</sup> Control hatch numbers were 58, 56, 54 (per 100 eggs)<sup>c</sup> Numbers in parentheses represent % WSF by volume in dilution.

TABLE 32

n-HEXANE WSF, i-OCTANE WSF, AND TETRALIN WSF - Artemia salina HATCHABILITY BIOASSAYS

<u>n</u> -Hexane			<u>i</u> -Octane			Tetralin		
Log (Hydrocarbon Conc., ppm w/v)	Relative Hatchability %		Log (Hydrocarbon Conc., ppm w/v)	Relative Hatchability %		Log (Hydrocarbon Conc., ppm w/v)	Relative Hatchability %	
0	100 <sup>a</sup>		0	100 <sup>b</sup>		0	100 <sup>c</sup>	
-0.19(7) <sup>d</sup>	88		-0.60(18) <sup>d</sup>			0.79(18) <sup>d</sup>	33	
0.03(11.7)	86		-0.46(25)			0.93(25)	10	
0.25(19.4)	94		-0.30(35.5)			1.09(35.5)	0	
0.47(32.4)	10		-0.15(50)			1.24(50)	0	
0.70(54.0)	0		0.0(71)			1.39(71)	0	
0.92(90.0)	0		0.15(100)			1.54(100)	0	

<sup>a</sup> Control hatch numbers were 64, 57 (per 100 eggs)

<sup>b</sup> Control hatch numbers were 47, 55 (per 100 eggs)

<sup>c</sup> Control hatch numbers were 47, 55 (per 100 eggs)

<sup>d</sup> Numbers in parentheses represent % WSF by volume in the dilution

TABLE 33  
INDIVIDUAL HYDROCARBON TOXICITY (LC 50) VALUES  
(Based on Artemia hatchability bioassay)

<u>Hydrocarbon</u>	<u>Toxicity</u>		
	<u>LC 50</u> <u>ppm w/v</u>	<u>95 %</u> <u>Conf. Limits</u> <u>ppm w/v</u>	<u>Mean</u> <u>LC 50</u> <u>ppm ± 1 SD</u>
Benzene	71.1	13.0	66.3 ± 5.0
	60.5	13.0	
	63.7	13.6	
	69.8	13.9	
Toluene	34.8	3.5	39.9 ± 5.7
	38.8	1.3	
	46.1 <sup>a</sup>	3.9	
<u>n</u> -Pentane	4.6	0.03	4.9 ± 0.4
	5.2	0.03	
<u>n</u> -Heptane	1.68	0.04	1.5 ± 0.3
	1.3 <sup>a</sup>	0.02	
<u>n</u> -Hexane	2.1	0.01	
<u>i</u> -Octane	~ 1		
Tetralin	6.2 <sup>a</sup>	0.02	

<sup>a</sup> Value obtained by extrapolation — dilution range unsuitable for normal moving average computation.

<sup>b</sup> Rough estimate only since 100 % WSF barely exceeds 50 % toxicity level.

supposedly low-toxicity alkane components of the JP-4 were examined. The results, displayed in Tables 30, 31, 32, and 33, were again surprising in that these aliphatic hydrocarbons proved to be much more toxic than expected. Comparison with the data for benzene and toluene showed that these straight and branched chain alkanes were as much as 15 to 50 times more toxic than the aromatics. This was a very interesting and significant result in view of the widely held and frequently expressed opinion (see above references) that aromatic hydrocarbons were largely responsible for the toxicity of petroleum fractions. Since in a typical jet fuel WSF these aromatics (benzene, toluene, xylenes) contributed only ~ 20 % of the toxicity (while comprising ~ 80 % of the WSF), it was evident that the contribution of the remaining hydrocarbons, including the very toxic and quite soluble lower alkanes, might be quite substantial. It certainly appeared feasible on the basis of this surprisingly high alkane toxicity to account for the overall toxicity of jet fuel in terms of its hydrocarbon components without having to invoke highly toxic non-hydrocarbon components such as additives.

#### TOXICITY OF MODEL HYDROCARBON MIXTURES

In the previous section we discussed our findings regarding the unexpectedly low toxicity of the major JP-4 WSF components, the aromatics benzene and toluene. The equally surprising high toxicity for the alkanes provided one explanation for the high (relative to benzene and toluene) JP-4 toxicity. However, it was also possible that the high JP-4 toxicity might be at least in part a synergistic effect of the hydrocarbons in combination. This possibility was explored by the preparation and testing of "synthetic" JP-4 WSF's consisting of some of the major hydrocarbons or minor hydrocarbons representative of different hydrocarbon classes. These included the benzene/toluene/ethylbenzene/xylenes mixture and the naphthalene/tetralin/decalin/benzene mixture whose preparation and analyses were described in an earlier section (page 41). To ensure that the components of these mixtures would be exerting the same effect as in the "real" JP-4 (GEC-1A-792033), the WSF compositions were adjusted to correspond as closely as possible with their levels in the JP-4 WSF (starting, however, at 300 % of the "equivalent" JP-4 WSF level to "catch" possible low toxicity readings). The bioassay data are summarized in Tables 34 and 35, and the toxicities in Table 36.

TABLE 34

BENZENE/TOLUENE/ETHYLBENZENE/XYLENES MIXTURE<sup>a</sup>  
 ("SIMULATED" JP-4 GEC-1A-792033 WSF) --  
Artemia salina HATCHABILITY BIOASSAY

Conditions: eggs, 100/dilution; incubation temperature 26° C

WSF Conc. ppm Total HC	Relative Hatchability, %	
	Series I	Series II
0	100 <sup>b</sup>	100 <sup>b</sup>
4.1 (20) <sup>c</sup>	107	111
8.2 (40) <sup>d</sup>	95	90
10.3 (50)	99	103
12.3 (60)	86	101
20.6 (100)	86	86
30.8 (150)	86	93
41.4 (200)	61	50
51.4 (250)	15	12
61.8 (300)	0	0

<sup>a</sup> Composition: benzene 31.8 ppm; toluene 20.4 ppm; ethylbenzene 1.65 ppm; m- + p-xylenes 5.31 ppm; o-xylene 2.49 ppm

<sup>b</sup> Control hatch numbers were 66, 69 (per 100 eggs).

<sup>c</sup> Numbers in parentheses indicate equivalent JP-4 (GEC-1A-792033) % WSF by volume.

<sup>d</sup> The 50 % WSF data were obtained by interpolation based on a least squares plot of the other data.

The results with both mixtures showed no evidence for a substantial synergistic effect. In both cases the LC 50 concentration was at least two times the maximum attainable concentration (100 % WSF) in the actual JP-4 WSF. With regard to the benzene/toluene/ethylbenzene/xylenes mixture, the LC 50 (expressed in terms of total hydrocarbon) at ~40 ppm indicated a toxicity about 5-6 times lower than the JP-4 (LC 50 = 7.7 ppm (Table 26) ). Thus, these aromatic compounds, comprising some 80-90 % of the WSF appeared to contribute less than 20 % of the toxicity. This conclusion was comparable to those based on benzene alone and therefore lent no support to a synergistic mechanism to explain the JP-4 toxicity.

TABLE 35

NAPHTHALENE/TETRALIN/DECALIN MIXTURE<sup>a</sup> (WITH BENZENE)

("SIMULATED" JP-4 GEC-1A-792033 WSF) -

Artemia salina HATCHABILITY BIOASSAY

Conditions: eggs, 100/dilution; incubation temperature 26° C

WSF Concentration		Relative Hatchability, %	
ppm Total HC	ppm Benzene	Series I	Series II
0	0	100 <sup>b</sup>	100 <sup>b</sup>
6.1 (20) <sup>c</sup>	6.0	99	99
11.3 (40) <sup>d</sup>	11.1	86	100
14.4 (50)	14.1	87	91
17.4 (60)	17.1	80	88
28.7 (100)	28.1	74	87
43.5 (150)	42.7	66	63
58.5 (200)	57.1	66	64
72.3 (250)	70.8	42	47
87.1 (300)	85.3	25	13

<sup>a</sup> WSF composition: naphthalene 1.35 ppm; tetralin 0.115 ppm; decalin, trans-isomer 0.083, cis-isomer 0.215; benzene 85 ppm.

<sup>b</sup> Control hatch numbers were 72, 80 (per 100 eggs)

<sup>c</sup> Numbers in parentheses indicate equivalent JP-4 (GEC-1A-792033) % WSF by volume.

<sup>d</sup> The 50 % data were obtained by interpolation.

The naphthalene/decalin/tetralin/benzene mixture was concocted to explore the possibility that some of the minor WSF components of higher molecular weight and with structural differences (bicyclic, cycloparaffins, etc.) might have an unusually high toxicity, alone or in synergistic combination, that would be sufficient to account for the "missing" major part of the JP-4 toxicity. Again the composition corresponded with component levels in JP-4 GEC-1A-7029033. As noted earlier (page 44), the toxicity of the benzene which was present to facilitate the naphthalene dissolution was already known and in any case was not expected to obscure any substantial



TABLE 36  
TOXICITY OF SIMULATED JP-4 (GEC-1A-792033) WSF's

Simulated WSF	LC 50		LC 50	
	% WSF <sup>a</sup>	95 % Confidence Limits	ppm Total HC	95 % Confidence Limits
Benzene/Toluene/ Ethylbenzene/Xylenes <sup>b</sup>				
Test Series I:	198.7	5.0	40.9	1.0
Test Series II:	195.7	4.7	40.3	1.0
Naphthalene/Tetralin/ Decalin/Benzene <sup>c</sup>				
Test Series I:	229	32.1	65.7	9.2
Test Series II:	225	26.0	64.6	7.5

<sup>a</sup> Equivalent JP-4 (GEC-1A-792033) % WSF by volume

<sup>b</sup> See Table 17 for composition of WSF.

<sup>c</sup> See Table 19 for composition of WSF.

synergistic effects of the other three components. The data (Tables 35 and 36) gave an LC 50 value of ~ 65 ppm total hydrocarbon which corresponded closely with the value for pure benzene WSF (66.3 ppm). This was the expected result if the toxicities of naphthalene, decalin, and tetralin were "normal," i.e. not exceptionally high, since benzene overwhelmingly predominated (98 %) in the simulated WSF. However, if any of the three test components had been a major contributor to JP-4 toxicity, at their level in the LC 50 dilution (equivalent to 230 % JP-4 WSF) of the simulated WSF, the Artemia hatch level would have been much lower than 50 %. The conclusion therefore was that none of these hydrocarbons was the "major toxicant" in JP-4.

The above two "simulated" JP-4 WSF's had provided no evidence for synergism that would explain the high JP-4 toxicity. A more direct test of the additivity of hydrocarbon toxicities was desirable, especially one involving

aliphatic hydrocarbons which had not hitherto been evaluated in mixtures. Therefore, another series of experiments was run using "synthetic fuel" mixtures containing two aromatics, benzene and toluene, and two alkanes, n-pentane and n-heptane. These lower boiling alkanes were selected because the very low solubilities of individual alkanes in the median JP-4 range precluded LC 50 determinations. (It should be noted that this does not necessarily mean that their toxic contribution to the WSF is negligible since the toxicity (of both alkanes and aromatics) rises with increasing molecular weight and the cumulative effect of their large numbers may be considerable). Mixtures containing known amounts of the four hydrocarbons were used to prepare WSF's which were bioassayed (Table 37). Based on the analyzed composition (Table 20) of the WSF's and the measured individual hydrocarbon toxicities (Table 33), predicted toxicities for the WSF's, assuming additivity, were calculated from the equation:

$$\begin{aligned} \text{LC 50 Mix (\% WSF)} = 100 \div & \left[ \frac{\underline{n} - C_5 \text{ in WSF, ppm}}{\text{LC 50 } \underline{n} - C_5 \text{ (ppm)}} \right. \\ & + \frac{\underline{n} - C_7 \text{ in WSF, ppm}}{\text{LC 50 } \underline{n} - C_7 \text{ (ppm)}} \\ & + \frac{\text{benzene in WSF, ppm}}{\text{LC 50 benzene (ppm)}} \\ & \left. + \frac{\text{toluene in WSF, ppm}}{\text{LC 50 toluene (ppm)}} \right] \end{aligned}$$

Agreement with experimentally determined WSF toxicities (Table 38) was excellent, supporting the additive nature of the toxic effect of these hydrocarbon mixtures. It is reasonable to conclude that a similar additivity applies to all the hydrocarbon components of JP-4 WSF.

TABLE 37

BENZENE/TOLUENE/n-PENTANE/n-HEPTANE WSF's -  
Artemia salina HATCHABILITY BIOASSAYS

Conditions: eggs, 100/dilution; incubation temperature 26° C

WSF Concen- tration % v	Relative Hatchability, %			
	Composition A <sup>a</sup>			Composition B <sup>b</sup>
	WSF I <sup>c</sup>	WSF II <sup>c</sup>	WSF III <sup>c</sup>	WSF I <sup>c</sup>
0	100 <sup>d</sup>	100 <sup>e</sup>	100 <sup>f</sup>	100 <sup>g</sup>
5	85	92	98	-
10	77	80	108	89
15	77	75	83	99
20	53	41	50	69
25	9	13	5	47
30	0	0	0	15
35	0	0	0	0
40	-	-	-	0

<sup>a</sup> Composition of neat hydrocarbon mixture by volume: benzene 3 parts; toluene 3 parts; n-pentane 16 parts; n-heptane 44 parts.

<sup>b</sup> Composition of neat hydrocarbon mixture by volume: benzene 3 parts; toluene 6 parts; n-pentane 25 parts; n-heptane 130 parts.

<sup>c</sup> For analyzed WSF composition see Table 20.

<sup>d</sup> Control hatch numbers: 52, 46, 65 (per 100 eggs)

<sup>e</sup> Control hatch numbers: 68, 63, 52 (per 100 eggs)

<sup>f</sup> Control hatch numbers: 60, 62, 58 (per 100 eggs)

<sup>g</sup> Control hatch numbers: 55, 68, 55 (per 100 eggs)

TABLE 38  
TOXICITY OF MODEL AROMATIC/ALKANE MIXTURES (BENZENE,  
TOLUENE, n-PENTANE, n-HEPTANE) - COMPARISON OF  
PREDICTED AND MEASURED LC 50 VALUES

Hydrocarbon Mixture <sup>a</sup>	LC 50, % WSF	
	Measured	Predicted
Composition A:		
WSF I	18.1	16.7
WSF II	17.6	16.2
WSF III	19.4	16.7
Composition B:		
WSF I	23.9	24.0

<sup>a</sup> See Table 20 for composition of hydrocarbon mixtures and analyses of the resulting WSF's.

#### SOURCE OF JP-4 TOXICITY AND THE CONTRIBUTION OF INDIVIDUAL HYDROCARBON COMPONENTS

Although little was known regarding the nature and source of the toxic components in JP-4 jet fuels when this investigation was started, it was hoped that the toxic activity might be traced to a single compound or at most to a limited number of components, possibly even among the several non-hydrocarbon additives that may be included in the fuel for a variety of reasons (de-icing agents, anti-oxidants, etc.). It then might be possible to eliminate or at least minimize the toxic effects of a fuel spill by omitting or reducing the appropriate components. The step-by-step investigations, detailed in the preceding sections have led to the following conclusions:

1. The major WSF components, the aromatic hydrocarbons benzene, toluene, and the xylenes, were all toxic, as expected from previous work. However, because of their relatively low individual toxicities and in the absence of synergistic effects (the latter discounted by our tests with hydrocarbon mixtures) these hydrocarbons obviously could not account for the toxic levels of JP-4 WSF's.

2. Alkanes were substantially more toxic than aromatic hydrocarbons of similar molecular weight or carbon number. As a result, despite their much lower water solubility, their toxic contributions could very well be comparable.

In light of the above conclusions a crucial question was how much of the JP-4 toxicity was still unaccounted for. In order to come to a reasonably definite conclusion on this point, it was necessary to derive a number of toxicity estimates since the complete analysis and toxicity of every hydrocarbon WSF component was beyond current capabilities. We estimated the toxicity of certain hydrocarbon fuel components on the basis of measured data and the presumption (supported by our results with model mixtures (page 59)) of the additivity of individual toxicities and compared the results with realistic expectations for these materials. Exceptionally high (or low) toxicities out of line with our experimentally determined trends in aromatic and aliphatic toxicities would then imply toxic contributions from different and probably non-hydrocarbon sources.

Two fuel components were of particular interest, the ethylbenzene/xylenes group of aromatic hydrocarbons and the "residual" hydrocarbons representing the remaining unmeasured hydrocarbons in the JP-4 WSF. An estimated toxicity for the xylenes group was a necessary intermediate goal since they could then be excluded from the 'residuals' group which would comprise all the saturated (aliphatic) hydrocarbons plus the remaining low level higher aromatics (bicyclics, etc.) and cycloparaffins. This 'residuals' group was of primary interest since its estimated toxicity could either support or refute the hypothesis of hydrocarbons as the sole source of JP-4 toxicity.

The basis for estimated toxicities was the general equation:

LC 50 (of component X)

$$= \frac{\text{LC 50 (of H in terms of component X)}}{1 - \frac{\text{LC50 (of H in terms of component C}_1\text{)}}{\text{LC50 (of pure component C}_1\text{)}} - \frac{\text{LC50 (of H in terms of component C}_n\text{)}}{\text{LC50 (of pure component C}_n\text{)}}}$$

where:

X = any component or mixture of components not included in

$C_1 \dots C_n$

$C_1 \dots C_n$  = components of known toxicity in hydrocarbon mix H

H = hydrocarbon mixture consisting of components  $C_1 \dots C_n$  and X.

The measured LC 50 values that were used in the calculations are collected in Table 39.

The basis for the estimate of ethylbenzene/xylenes toxicity was the measurements on the benzene/toluene/xylenes "simulation" mix (see Table 39 and page 59 ) for JP-4 (GEC-1A-792033). In this case the equation became:

LC 50 (of ethylbenzene/xylenes)

$$= \frac{\text{LC50 (of "simulated" JP-4 mix in terms of xylenes/ethylbenzene)}}{1 - \frac{\text{LC50 (of simulated JP-4 mix in terms of benzene)}}{\text{LC50 (of pure benzene)}}}$$

$$\dots \frac{\text{LC50 (of simulated JP-4 mix in terms of toluene)}}{\text{LC50 (of pure toluene)}}$$

$$= \frac{6.09}{1 - \frac{21.0}{66.3} - \frac{13.9}{39.9}}$$

$$= 18.2 \text{ ppm w/v}$$

This toxicity estimate for the xylenes group was in the expected range based on the measured values for and the increasing toxicity of the lower series members, benzene (66.3 ppm w/v) and toluene (39.9 ppm w/v).

Since the composition of the above "simulated" WSF was identical to the real JP-4 (GEC-1A-792033) in terms of these aromatic components, it was now possible to estimate the toxicity of the remaining components ("residuals") in the real JP-4. This time the equation became:

TABLE 39  
MEASURED LC 50 VALUES

<u>Component</u>	<u>LC 50 ppm w/v</u>
Benzene	67.1
Toluene	34.4
Aromatic Mix <sup>a</sup> ("simulated" JP-4):	
a) basis total hydrocarbons (HC)	40.65
b) basis benzene	21.0
c) basis toluene	13.45
d) basis xylenes/ethylbenzene	6.1
JP-4 (GEC-1A-792033):	
a) basis total HC	9.1
b) basis "residuals" HC	1.9
c) basis benzene	3.6
d) basis toluene	2.4
e) basis xylenes/ethylbenzene	1.18
JP-4 (Arco JP-4-42-81):	
a) basis total HC	10.5
b) basis "residuals" HC	1.4
c) basis benzene	1.42
d) basis toluene	5.2
e) basis xylenes/ethylbenzene	2.4
JP-4 (Friendswood, Batch 66, Tank 651):	
a) basis total HC	13.5
b) basis "residuals" HC	1.6
c) basis benzene	6.18
d) basis toluene	3.7
e) basis xylenes/ethylbenzene	2.0
JP-4 (Exxon, 81-894):	
a) basis total HC	12.3
b) basis "residuals" HC	1.54
c) basis benzene	3.9
d) basis toluene	3.3
e) basis xylenes/ethylbenzene	3.6
JP-4 (Shale, MS-001-792186):	
a) basis total HC	7.5
b) basis "residuals" HC	2.13
c) basis benzene	1.13
d) basis toluene	2.60
e) basis xylenes/ethylbenzene	1.66

<sup>a</sup> Analysis: benzene 31.8 ppm w/v, toluene 20.4 ppm w/v, ethylbenzene 1.65 ppm w/v, m- + p-xylenes 5.31 ppm w/v, o-xylene 2.49 ppm w/v

LC 50 (of "residuals")

$$= \frac{\text{LC50 (of JP-4 WSF in terms of "residuals")}}{1 - \frac{\text{LC50 (of WSF in terms of benzene)}}{\text{LC50 (of pure benzene)}}}$$

$$\dots \frac{\text{LC50 (of WSF in terms of toluene)}}{\text{LC50 (of pure toluene)}} -$$

$$\dots \frac{\text{LC50 (of WSF in terms of xylenes/ethylbenzene)}}{\text{LC50 (of pure xylenes/ethylbenzene)}}$$

$$= \frac{1.9}{1 - \frac{3.6}{66.7} - \frac{2.4}{39.9} - \frac{1.18}{18.2}}$$

$$= 2.3 \text{ ppm w/v}$$

The calculation was extended to the other JP-4 samples using the appropriate data from Table 39. Since data for the specific xylenes mix composition in each JP-4 WSF were not available, the above estimate for GEC-1A-792033 was assumed for all JP-4's. This appeared unlikely to distort the results appreciably since LC 50 values for individual xylenes were found to be similar (o-xylene 15.0 ppm, m-xylene 16.5 ppm, p-xylene 149 ppm, ethylbenzene 12.4 ppm) and therefore group composition changes would have little effect. Moreover, variations in the xylenes group LC 50 from 15 ppm to 25 ppm, for example, only changed the "residuals" LC 50 from 2.29 to 2.38 ppm. A summary of the calculated residuals LC 50's is given in Table 40.

As can be seen from the table, the toxicities of the "residuals" were strikingly similar in all the JP-4's. Even more significant, the toxicity values were in the same range as our measured pure alkanes, e.g. n-pentane 4.9 ppm, n-hexane 2.1 ppm, n-heptane 1.5 ppm. Thus it would appear that hydrocarbon and in particular alkane toxicities were in the right range to account for the "residuals" JP-4 toxicity without the need to invoke toxic effects from non-hydrocarbon sources in the fuel.



The general conclusion, therefore, was that although the overall toxicity of the five different JP-4 jet fuels varied over a 2-3 fold range, in every case the toxicity could be accounted for by the cumulative effect of the hydrocarbon components. The unfortunate corollary to this is that it is not possible to reduce JP-4 toxicity by the elimination or reduction of a few key components.

TABLE 40  
ESTIMATED LC 50 VALUES FOR "RESIDUALS" IN WSF's FROM  
DIFFERENT JP-4 SAMPLES

<u>JP-4</u>	<u>"Residuals" LC 50</u> <u>ppm w/v</u>
GEC-1A-792033	2.3
Arco (JP4-4281)	2.0
Friendswood (Batch 22, Tank 651)	2.3
Exxon (81-894)	2.6
Shale (MS-000)-792186)	2.3

Since hydrocarbons contributed additively to the overall fuel toxicity, it was possible to assess individual hydrocarbon contributions, provided the toxicity of the "pure" hydrocarbon and the amount in the JP-4 WSF were known. The appropriate equation is:

Individual Toxic Contribution of Hydrocarbon (TCH) No.

$$= \frac{\text{LC 50(of JP-4 WSF in terms of individual hydrocarbon), ppm}}{\text{LC 50(of individual hydrocarbon), ppm}} \times 100 \%$$

When applied to the synthetic hydrocarbon mixture (A) in Table 20, the results shown in Table 41 were obtained. These figures were calculated on the basis of measured LC 50 values and their sum, which should equal 100 %, diverges from the latter to the extent that the measured and calculated LC 50's differ. The significant conclusion was that even in a hydrocarbon mixture where the WSF aromatics greatly exceed the alkanes (94 % to 6 %), the alkanes still contributed half the toxicity.

TABLE 41  
INDIVIDUAL TOXICITY CONTRIBUTION OF HYDROCARBONS (ITCH No.)  
IN A HYDROCARBON MIXTURE

	Composition of Mix WSF <sup>a</sup>		ITCH No. <sup>b</sup>
	ppm w/v	%	
Benzene	144.2	74.9	40
Toluene	37.1	19.3	17
n-Pentane	9.9	5.1	37
n-Heptane	1.44	0.7	18

<sup>a</sup> Combined mean for Mixes I, II, and III (Table 37)

<sup>b</sup> Measured LC 50 values used in ITCH No. computation: Mix (17.9% WSF); benzene (66.3 ppm w/v); toluene (39.9 ppm w/v); n-pentane (4.9 ppm w/v); n-heptane (1.5 ppm w/v).

The same procedure was used to calculate a set of ITCH Nos. for the hydrocarbons of the different JP-4 WSF's using measured LC 50 values for WSF's, n-pentane, benzene, and toluene and estimated LC 50's for the ethylbenzene/xylenes and the "residuals." While the results (Table 42) were approximate because of the use of estimated LC 50 values, they provided a useful indication of the extent of each hydrocarbon's contribution to the overall toxicity. One major conclusion was the smallness of the contribution made by the major WSF hydrocarbons (benzene, toluene, etc.) to all JP-4 samples regardless of their overall toxicity (i.e. "high" Friendswood or "low" Shale). No single hydrocarbon (or group), with the exception of the xylenes in the Exxon JP-4, contributed more than 10% to the total JP-4 toxicity.

TABLE 42  
INDIVIDUAL TOXICITY CONTRIBUTION OF HYDROCARBONS (ITCH No.)  
IN DIFFERENT JP-4 SAMPLES

	ITCH No., %				
	Monsanto (GEC-1A- 792033)	Arco (JP-4-42-81 Tank R40)	Friendswood (Batch 66 Tank 651)	Exxon (81-894)	Shale (MS-0001- 792186)
Benzene	5	2	9	6	2
Toluene	6	13	9	9	7
Ethylbenzene/ Xylenes	6	12	11	18	8
n-Pentane	6	2	4	5	9
"Residuals"	82	70	70	60	93

#### PREDICTION OF JP-4 TOXICITIES AND SOLUBILITIES

In this report WSF's from five different samples of JP-4 have been compared and shown to differ significantly both in hydrocarbon composition and in toxicity. These differences are a reflection of differences in the hydrocarbon composition of the neat fuels. Such differences have been demonstrated with regard to benzene and toluene (Table 13) which because of their relatively high solubility comprise a major portion of the WSF's.

The ability to predict JP-4 behavior with regard to toxicity and water solubility would be useful in a number of different ways. For example, it would be very convenient if an estimate of the toxicity of different JP-4's could be obtained without the time delays involved in preparing WSF's (24-48 hours) and bioassaying them (at least another 48 hours). It would also be very useful if toxicity could be predicted from a quick analysis of the water layer (WSF) under a spill, again without requiring an extended bioassay.

From a comparison of the five different JP-4 samples, it is evident that the toxicity, not unexpectedly, increases with increasing water solubility of the JP-4. Using our accumulated analytical and LC 50 data we have developed the following correlations.

1. WSF toxicity can be predicted from benzene + toluene levels in the neat JP-4. Linear regression analysis of log LC 50, as percent

WSF, versus log (percent benzene + toluene) in the neat fuel gave the line shown in Figure 11 with a correlation coefficient of 0.89. This indicates that at least within the range from shale JP-4 with a relatively low toxicity ( $LC_{50} = 46.3\%$  WSF) to the Friendswood JP-4 which is about three times as toxic, it is possible to predict the maximum toxicity of the WSF from these jet fuels. Unfortunately, the 95 % confidence limits are quite wide, presumably reflecting the inevitable variability of biological data and probably also the fact that the computation is based on only two of the many WSF components in the fuel. The fact that these two aromatics can be used at all to predict toxicity reflects the substantial effect that changes in their proportions in the neat fuel can have on their toxic contribution to the WSF. Cautious extrapolation of the data to the maximum allowable JP-4 aromatic hydrocarbon level (25 %) suggests an upper toxicity limit of  $\sim 7\%$  WSF ( $LC_{50}$ ) for JP-4's.

2. WSF toxicity can be predicted from the total hydrocarbon in the WSF (Figure 12). As might be expected, the correlation is better here with a coefficient of 0.968. This correlation should prove useful in predicting the toxicity of water under or near a fuel spill from a quick GC analysis of a sample without having to wait several days for bioassay results.
3. Maximum JP-4 water solubility can be predicted from the benzene plus toluene levels in JP-4 (Figure 13). This follows from the above relationships. The linear regression line for log (total WSF hydrocarbons) versus log (percent benzene plus toluene in JP-4) has a positive correlation coefficient of 0.965. This relationship could be used to predict the maximum hydrocarbon concentration to be expected in water in contact with a given jet fuel. The plot, for example, predicts that the maximum possible JP-4 hydrocarbon concentration in water is  $\sim 300$  ppm (from a JP-4 with the specification maximum of 25 % benzene plus toluene).

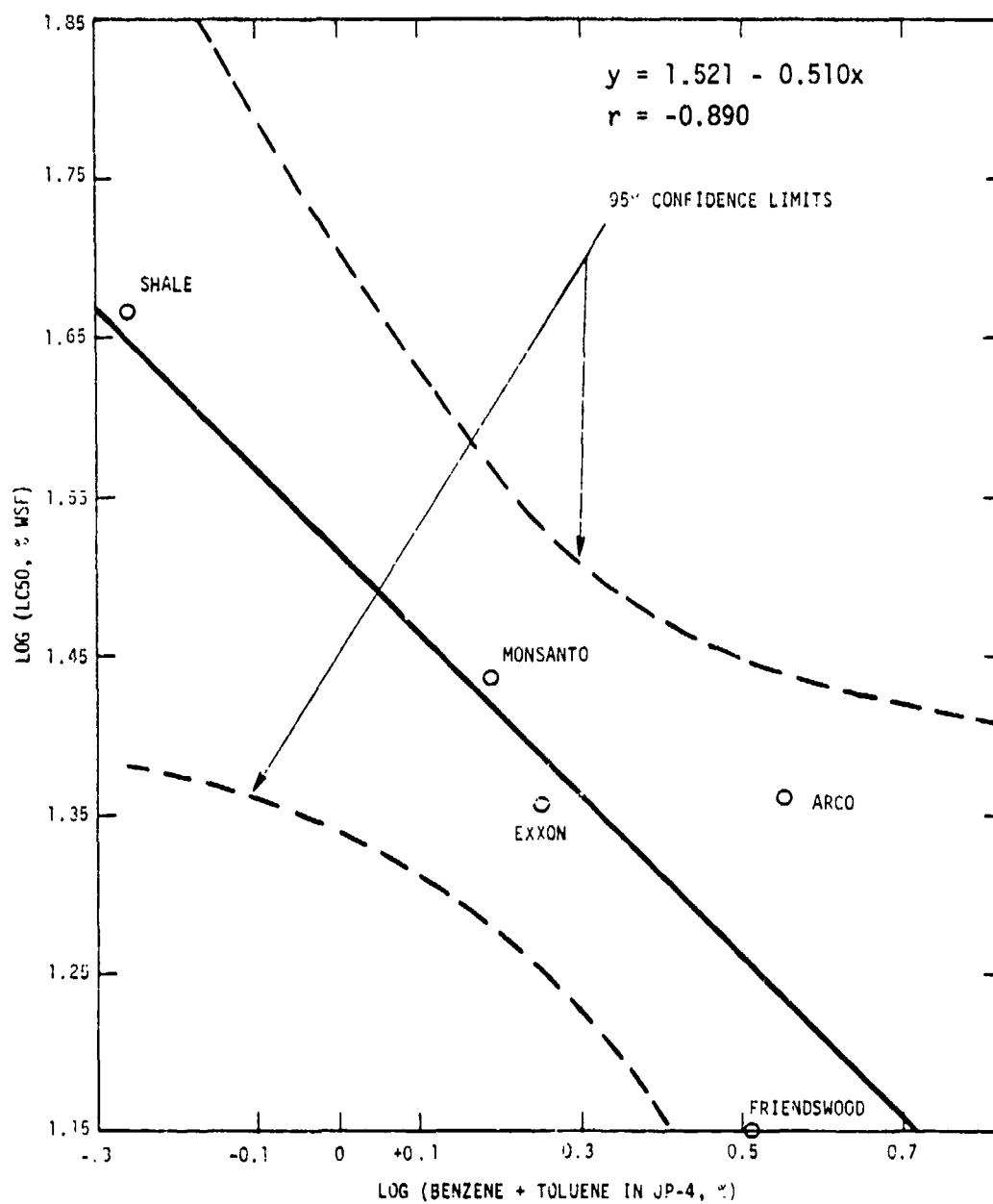


FIGURE 11. RELATIONSHIP BETWEEN BENZENE/TOLUENE LEVELS IN JP-4 AND THE TOXICITY OF JP-4 WSF

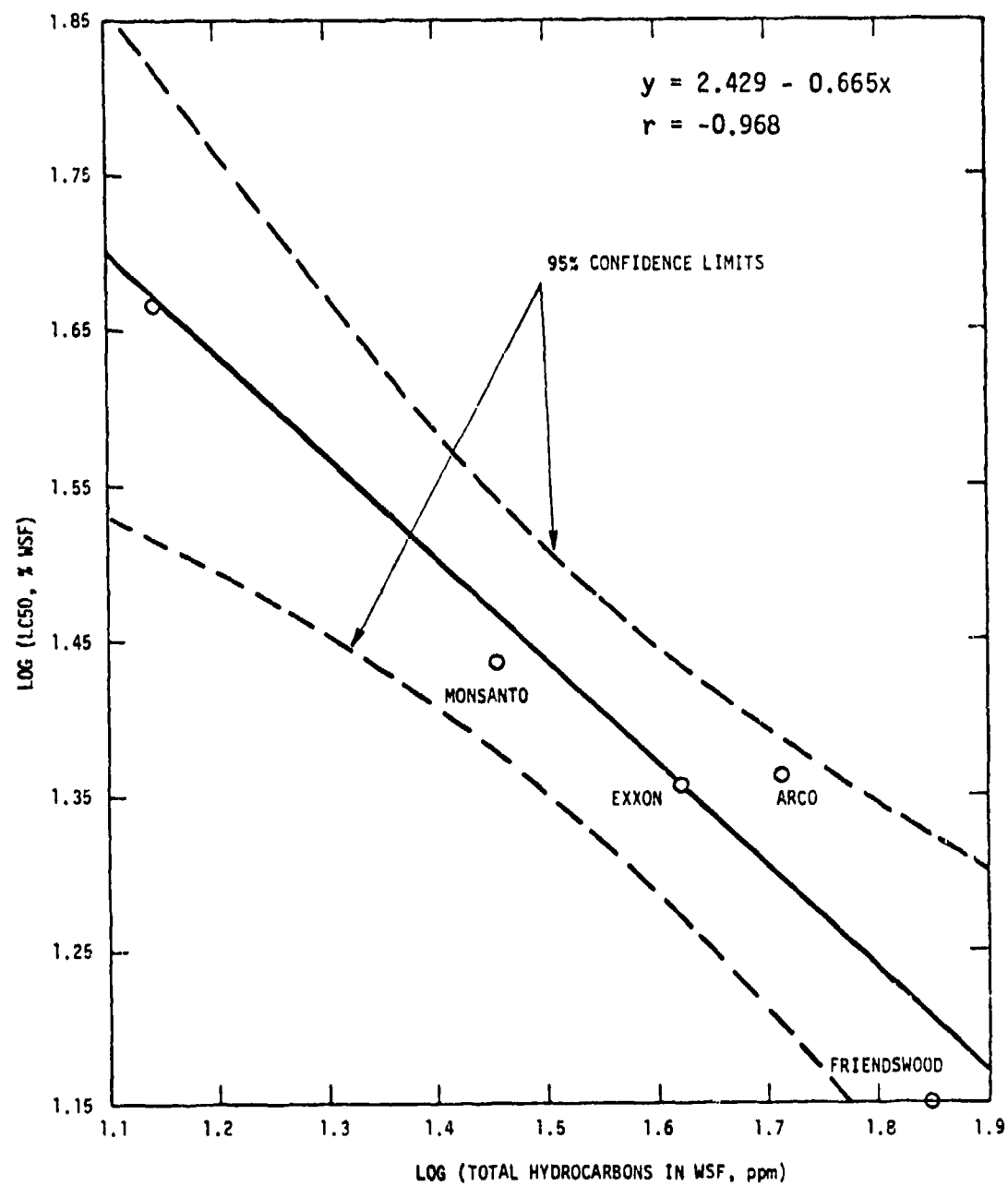


FIGURE 12. RELATIONSHIP BETWEEN TOTAL HYDROCARBON LEVEL AND TOXICITY IN JP-4 WSF

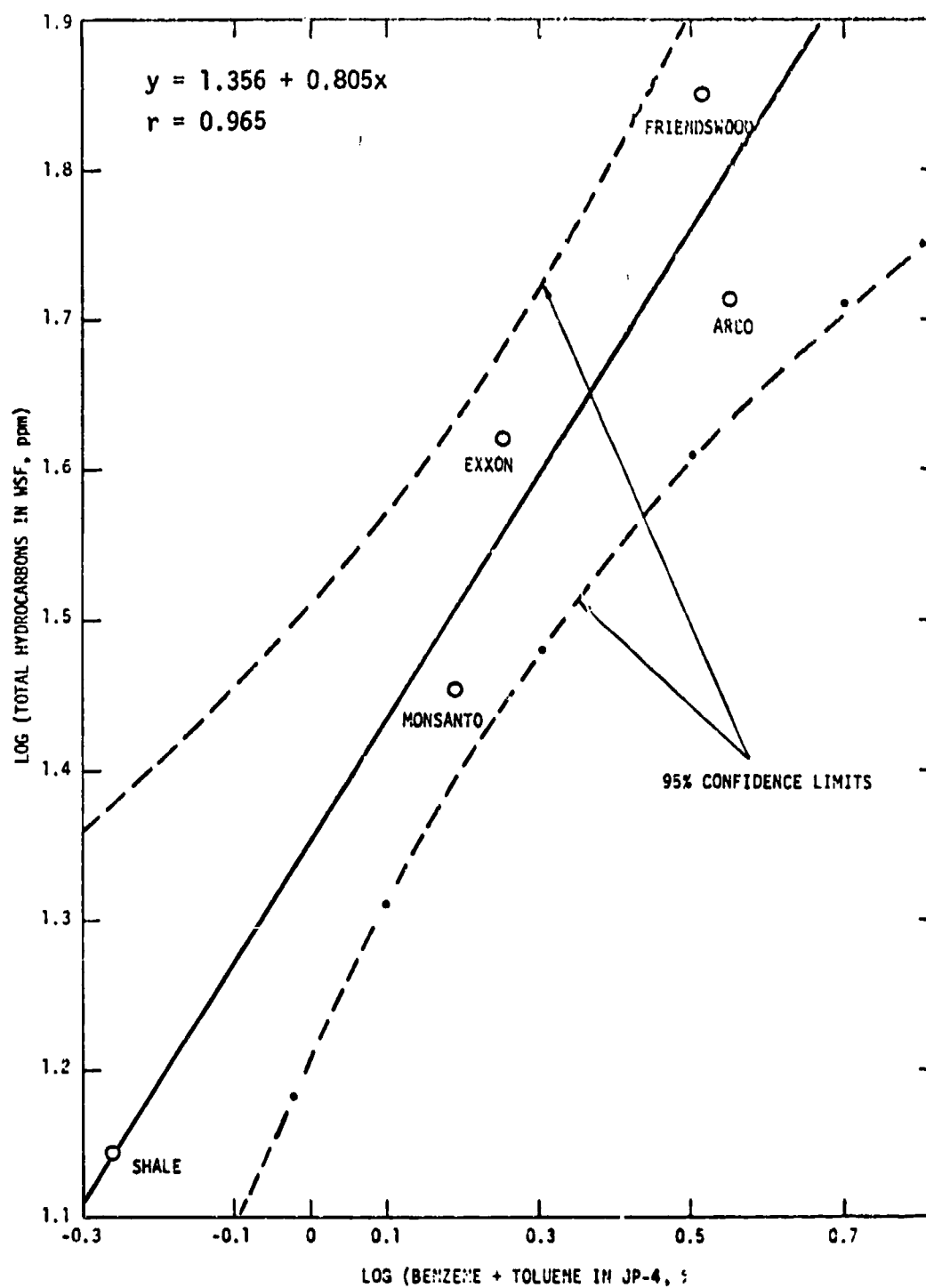


FIGURE 13. RELATIONSHIP BETWEEN BENZENE/TOLUENE IN JP-4 AND TOTAL HYDROCARBONS IN JP-4 WSF

It must be emphasized that the above relationships yield maximum numbers, i.e. maximum toxicities and maximum hydrocarbon levels for the WSF from a given JP-4, since all our data are derived from closed systems (deliberately so to avoid hydrocarbon losses during the bioassay test periods). There is every likelihood that in a real-life situation the dynamic nature of the fuel/water interaction will lead to substantially lower toxicities and concentration. Nevertheless, a knowledge of the maximum possible levels of soluble contaminants and toxicities could be of considerable importance in deciding what actions, if any, are needed in a particular spill situation, either chronic or acute.

Furthermore, it should be noted that all our toxicity data are based on the effect of JP-4 on the hatching of Artemia salina eggs. While the literature (Rice et al., 1976) suggests that the eggs of aquatic species are likely to be more tolerant to toxicants than larval or adult stages and therefore that our toxicity estimates are, if anything, on the conservative side, it would be prudent to confirm and expand these findings in other species and at other development stages.

#### INTERACTIVE EFFECTS OF AROMATIC AND ALIPHATIC HYDROCARBONS IN JP-4's

The evidence presented in this report strongly supports a cumulative explanation for the overall toxic impact of JP-4 jet fuels. Each hydrocarbon contributes its share to the toxicity in proportion to its specific toxicity and its concentration in the fuel. At first sight this relationship would appear to be straightforward since, in general, hydrocarbon toxicity increases and water solubility decreases with increasing molecular weight (carbon number). Unfortunately, the situation is confused from the point of view of visualizing the consequences of compositional changes by the large and conflicting differences between aromatic and aliphatic (saturated) hydrocarbons. On the one hand alkanes are much less soluble than the comparable (by carbon number) aromatic while with regard to toxicity the alkanes are much more toxic. Consequently, the effect of varying proportions of aromatics and alkanes on their relative contributions to the overall WSF toxicity is difficult to predict. The different effects can be illustrated by comparing the toxic contribution (ITCH No.) of



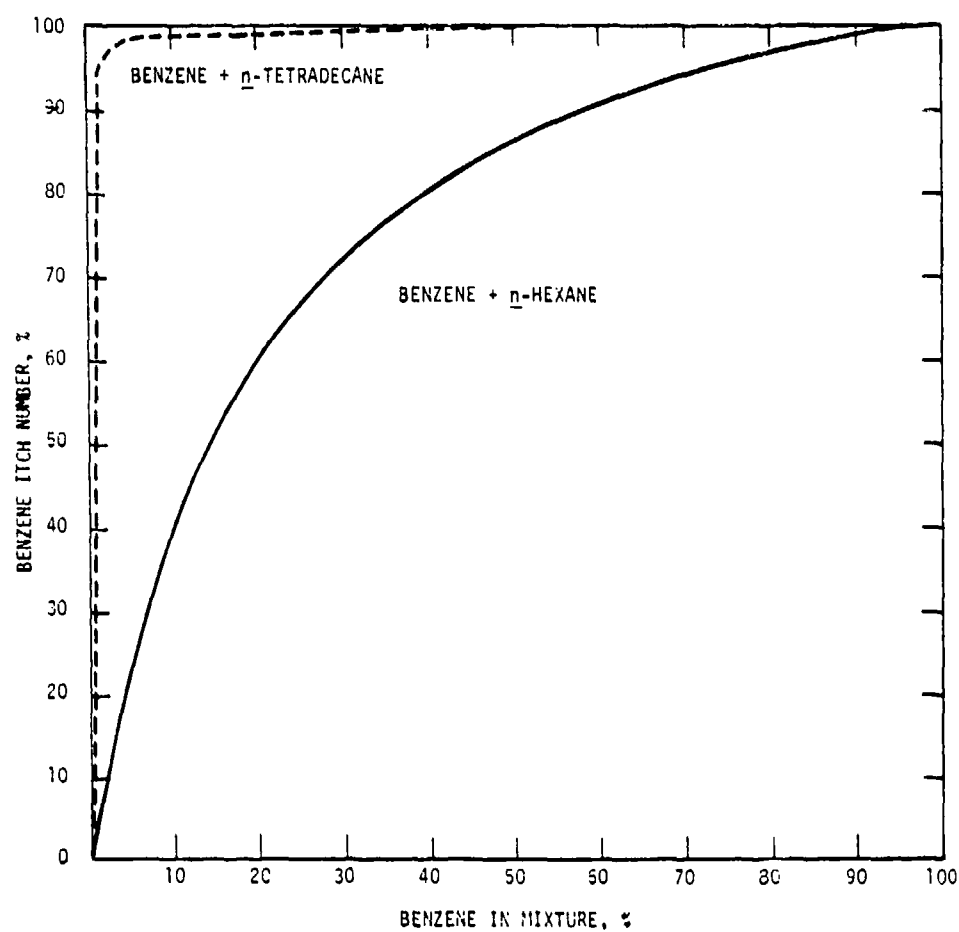


FIGURE 14. EFFECT OF ALKANE/AROMATIC COMPOSITION ON CONTRIBUTION TO WSF TOXICITY - COMPARISON OF BENZENE/n-HEXANE AND BENZENE/n-TETRADECANE MIXES  
 (Note: Benzene and n-hexane values are based on our measured data; n-tetradecane values are estimates from extrapolations of solubility and toxicity data of lower carbon number alkanes [pentane, hexane, heptane, octane])

benzene in binary mixtures with 1) a same carbon number alkane, n-hexane and 2) a higher carbon number alkane, n-tetradecane. As Figure 14 shows, in the WSF from the benzene/nC<sub>14</sub> binary mixture the toxicity is almost entirely due to the benzene in all compositions containing more than ~ 2 % benzene. On the other hand, the benzene contribution in mixtures with n-hexane is less than 50 % in mixtures containing up to 14 % benzene. In the 14 % benzene/86 % n-hexane mixture the toxicity of the resulting WSF derives equally from the aromatic and aliphatic components.

It is important to distinguish between the relative contributions to toxicity of different hydrocarbon classes discussed above and the actual toxicity of the WSF's. With regard to the latter it is enlightening to note that although benzene's intrinsic toxicity is low at ~ 66 ppm, its high solubility of ~ 1100 ppm (in 1.6 % Instant Ocean) means that its saturated solution is some fifteen times more concentrated than its LC 50 concentration whereas in the case of a highly toxic alkane such as n-hexane (LC 50 = 2.1 ppm) because of its low solubility (9.2 ppm) its saturated solution is only five times the LC 50 concentration. Therefore, from this point of view the potential toxicity of JP-4 fuels is limited by the specification maximum of 25 % aromatics. This effectively drops the maximum contribution of the major aromatics (benzene[ toluene[ xylenes) to some 10-30 % of the total toxicity of the WSF.

## APPENDIX A

### JP-4 ANALYSES SUPPLIED BY THE OIL COMPANIES

(Note: No data sheets available for the shale JP-4.)

# FINAL INSPECTION REPORT

CCN 9487 11-21-81

23 11

SAMPLE ENTRY DATE

11-21-81

SAMPLE IDENTIFICATION

T0411140

FORMULA

00073051K

GRADE

TURBO FUEL B

SOURCE

0509 SHIPPING TANK

AVG. GRAV. API @ 60 DEGS. F.

53.4

GRAVITY, SPECIFIC

0.7855

VACUUM PRESSURE REID

1.0

FREEZE POINT DEGS. F.

10.0

COLOR-SAYBOLT

30

CORROSION, CHRS. / 210 DEGS. F.

10.0

CRUDE POINT

WATER SEPARATION RATING

1

WATER INTERFERENCE RATING

0.2

GUM-EXTRACT STEAMING, 100 ML.

WILLINE POINT DEGS. F.

125.5

W.G. PRODUCT-CALC.

0000202

W.G. PRODUCT-CALC.

0.005

SULFUR CONTENT

0.010

MERCAPTAN SULFUR WTL

0.005

PERCENT AROMATICS BY FIA

00014.3

PERCENT OLEFINS BY FIA

00000.4

WSTM

78

PARTICULATE MATTER MG/LITER

0.1

AVG VACUUM, INCHES HG

23.9

FILTRATION TIME, MIN

14.13

TOTAL ACID DISEASING ROH/G

0.008

APPEARANCE AVG.

PASS

12010 REB DEGS. F. FOR REC.

0000.8

12020 CEN DEGS. C. FOR REC.

0113.0

12030 CEN DEGS. F. FOR REC.

0140.8

73 CEN FPA DEGS. C.

0129.0

DELIVER TYPE DD, VOP-M

0.1

UPTON TUNE RATING

1.0

WSTM NO. 501-11A

0.0

EXXON CO., USA, BATON ROUGE, LA.

CONTRACT NO. DLA-600-810-0441

DATE SAMPLED 11-21-81

TANK NO. 411

BATCH NO. 140

SAMPLING LOCATION - TANK

PRODUCT Turbo Fuel B

COMPLIES W/ SPEC-MIL-1555

APPROVED BY *[Signature]*  
11-25-81

EXXON

FUEL VOLATILITY

Sample FUEL 1A GEC-145-400-792033

Vol % Recovered	JP-4	JP-8	SFOLA 200 °C	MRC 78 °C	SFOLA 056 °C	SFOLA 056 °F	
IBP (0.5%)	*	+	28	2	60	140	10
1				34			
5			72	72			
10	*	++	89	93	97	206	10
15			97				
20	**	+	111	108	110	230	20
25			115				
30			119	123			30
35			126				
40			137	141			40
45			144				
50	**	+	155	160	146	295	50
55			166				
60			176	180			60
65			188				
70			197	199			70
75			208				
80			217	218			80
85			228				
90	**	+	237	237	224	435	90
95			253	254			
99				273			
FDP (99.5%)	**	++	283	281	248	478	90
Residue (%)	**	++				1.0	
Loss (%)	**	++				1.0	
Recovery at 400°F (201.1°C)						82.0	
Vapor Pressure (10 Held)	**				2.5		
Flash pt (°C)		++	D56 (Tag Closed)	D93 (Pensky-Martens)	D3243 (Solaflash)		
True Vapor Pressure (MRC) (mm Hg)			-20°F	+32°F	+70°F	100°F	
				10.0	82.5	116.0	

"STANDARD"  
Monsanto-supplied

FUEL VOLATILITY

Sample FUEL 1A GEC-145-400-792033

Vol % Recovered	JP-4	JP-8	SFOIA 2087 °C	MRC 2407 °C	SFOIA 086 °C	086 °F	
IBP (0.5%)	*	+	<u>28</u>	<u>25</u>	<u>60</u>	<u>140</u>	100
1				<u>34</u>			
5			<u>72</u>	<u>72</u>			
10	*	++	<u>89</u>	<u>93</u>	<u>97</u>	<u>206</u>	10
15			<u>97</u>				
20	**	+	<u>104</u>	<u>108</u>	<u>110</u>	<u>230</u>	20
25			<u>115</u>				
30			<u>119</u>	<u>123</u>			30
35			<u>126</u>				
40			<u>137</u>	<u>141</u>			40
45			<u>144</u>				
50	**	+	<u>155</u>	<u>160</u>	<u>146</u>	<u>295</u>	50
55			<u>166</u>				
60			<u>176</u>	<u>180</u>			60
65			<u>188</u>				
70			<u>197</u>	<u>199</u>			70
75			<u>208</u>				
80			<u>217</u>	<u>218</u>			80
85			<u>228</u>				
90	**	+	<u>237</u>	<u>237</u>	<u>224</u>	<u>435</u>	90
95			<u>253</u>	<u>254</u>			
99				<u>273</u>			
FDP (99.5%)	**	++	<u>283</u>	<u>281</u>	<u>248</u>	<u>478</u>	FDP
Residue (%)	**	++				<u>1.0</u>	
Loss (%)	**	++				<u>1.0</u>	
Recovery at 400°F (204.4°C)						<u>82.0</u>	
Vapor Pressure (14.7 in Hg)						<u>2.5</u>	
Flash pt (°C)		++					
			DC6 (Tag Closed)	DD3 (Penalty-Flare)	DD343 (Setback)		
True Vapor Pressure (MMG) (mmHg)			<u>-80°F</u>	<u>438°F</u>	<u>470°F</u>	<u>100°F</u>	
				<u>10.0</u>	<u>82.5</u>	<u>116.0</u>	

"STANDARD"  
Monsanto-supplied

Sample FUEL 1A GEC-145-400-792033

Color D156

+20

Acidity (mg KOH/g) D3242

0.002

Copper Strip (2 hrs at 212°F) D130

1A

Existent Gum (mg/100 ml) D381

0.8

Particulates (mg/l) D2276

0.1

Filtration Time D2276

17 min @ 27"

Water Reaction, Vol. Change (ml) D1094

0.0

Ratings

D1094

#1 #1

WSIM, Minisonic

96

Additives

1. Anti-icing (Vol %)
2. Antioxidant (lb/M Bbl)
3. Corrosion Inhibitor (lb/M Bbl)
4. Metal Deactivator (lb/M Bbl)
5. Antistatic (ppm)

.07

Monsanto-supplied  
JP-4

Analyses by SFQLA

VN-80-41

Sample FUEL-1A GEC-145-400-792033

79-F-2270

Data on Monsanto-supplied JP-4

79-F-2451

Composition

45  
45A  
09

	Vol %	
	Mass spec NRC	SFQLA
1. Paraffins	<u>61.2</u>	
2. Monocycloparaffins	<u>24.2</u>	
3. Dicycloparaffins	<u>4.9</u>	
4. Alkylbenzenes	<u>8.2</u>	
5. Indans and Tetralins	<u>1.1</u>	
6. Indenes and Dihydronaphthalenes	<u>—</u>	
7. Naphthalenes	<u>0.4</u>	
Aromatics (4 + 5 + 6 + 7) D1319	<u>9.7</u>	<u>12.3</u>
Olefins D1319		<u>1.5</u>
Total Paraffins (1+2+3)/D1319(100%-A-O)	<u>90.3</u>	<u>86.2</u>
-----		
Hydrogen Content (wt %)		
POSF - D3701 / SFQLA - D3343	<u>14.48</u>	<u>14.46</u>
Sulfur, Mercaptan (wt %) D1219-61		<u>0.0004</u>
Total (wt %) D2622-67		<u>0.03</u>
Net Heat of Combustion (BTU/lb)		
(NRC-D240/SFQLA-D3330)	<u>18767</u>	<u>18747</u>
Luminometer Number D1740		<u>77</u>
Smoke Pt. (SFQLA Calc/SFQLA-D1322)	<u>28</u>	<u>25.0</u>



# ARCO

DATE: 10/20/81 (JP-4-42-81) File: 526.2

ITEM NO.	APPEARANCE	RESULTS	SPEC LIMIT	REPORT DATE
100	Color (Black)	1.8	Report	October 27, 1981
200	Color (Black and Clear)	0.5	Report	CONTRACT NO. DLA-600-81 -D- 0353
COMPOSITION				DATE NO. 6024
100	Acidity, Total (mg KOH/g)	0.025	0.015 max	DATE SAMPLED 10/20/81
110	Arsenic (ppm)	0.05	25 max	SAMPLE NO.
120	Barium (ppm)	0.05	5 max	
130	Boron (ppm)	0.05	0.00 max	
140	Cadmium (ppm)	0.05	Report	
150	Copper (ppm)	0.05	0.40 max	
VOLATILITY				BATCH NO. JP-4-42-81
200	Distillation Initial BP (C)	1.0	Report	TANK NO. R-40
210	10% Res (C)	1.0	145 max	QUANTITY U.S. GALLONS
220	20% Res (C)	1.0	190 max	
230	30% Res (C)	1.0	245 max	
240	40% Res (C)	1.0	270 max	SAMPLING LOCATION ARCO - Watson Refinery
250	50% Res (C)	1.0	270 max	1801 E. Sepulveda Blvd
260	60% Res (C)	1.0	270 max	Carson, Ca. 90745
270	70% Res (C)	1.0	270 max	PRODUCT NAME TURBINE FUEL, AVIATION
280	80% Res (C)	1.0	270 max	COMPLIES WITH SPECIFICATION
290	90% Res (C)	1.0	270 max	
300	100% Res (C)	1.0	270 max	
310	Flash Point (F)	1.0	145 max	MIL-T-5624L Am. 1
320	Flash Point (C)	1.0	145 max	DESTINATION
330	Flash Point (F)	1.0	145 max	DFSP - Norwalk Ca.
340	Flash Point (C)	1.0	145 max	Via P/L ARCO
EMULSION				ANALYST JP-4
400	Antimony (ppm)	0.05	20 max	REMARKS
410	Asbestos (ppm)	0.05	20 max	* CONTAINS NO PSII
420	Barium (ppm)	0.05	20 max	** Ordered without conductivity additive
430	Boron (ppm)	0.05	20 max	Distribution:
440	Cadmium (ppm)	0.05	20 max	COMMANDER
450	Copper (ppm)	0.05	20 max	Air Force Systems
460	Cobalt (ppm)	0.05	20 max	ATTN: AFAPL(SFF)
470	Chromium (ppm)	0.05	20 max	AF Aero Propulsion Lab
480	Iron (ppm)	0.05	20 max	Wright-Patterson AFB
490	Lead (ppm)	0.05	20 max	Ohio, 45433
500	Magnesium (ppm)	0.05	20 max	COMMANDER
510	Manganese (ppm)	0.05	20 max	San Antonio ALC
520	Nickel (ppm)	0.05	20 max	Relly AFB, Texas 78111
530	Phosphorus (ppm)	0.05	20 max	
540	Silicon (ppm)	0.05	20 max	
550	Sulfur (ppm)	0.05	20 max	
560	Tantalum (ppm)	0.05	20 max	
570	Tin (ppm)	0.05	20 max	
580	Tungsten (ppm)	0.05	20 max	
590	Zinc (ppm)	0.05	20 max	
600	Zirconium (ppm)	0.05	20 max	
ADDITIONALS				SEE REVERSE SIDE
610	Acidity, Total (mg KOH/g)	0.025	0.015 max	
620	Arsenic (ppm)	0.05	25 max	
630	Barium (ppm)	0.05	5 max	
640	Boron (ppm)	0.05	0.00 max	
650	Cadmium (ppm)	0.05	Report	
660	Copper (ppm)	0.05	0.40 max	
670	Cobalt (ppm)	0.05	20 max	
680	Chromium (ppm)	0.05	20 max	
690	Iron (ppm)	0.05	20 max	
700	Lead (ppm)	0.05	20 max	
710	Magnesium (ppm)	0.05	20 max	
720	Manganese (ppm)	0.05	20 max	
730	Nickel (ppm)	0.05	20 max	
740	Phosphorus (ppm)	0.05	20 max	
750	Silicon (ppm)	0.05	20 max	
760	Sulfur (ppm)	0.05	20 max	
770	Tantalum (ppm)	0.05	20 max	
780	Tin (ppm)	0.05	20 max	
790	Tungsten (ppm)	0.05	20 max	
800	Zinc (ppm)	0.05	20 max	
810	Zirconium (ppm)	0.05	20 max	
CYCLIC SAMPLING				Max H. Gwinn - Manager
QUALITY ADMINISTRATIVE				

CRUDE SOURCE: 80% high sulfur, predominately Alaskan  
North Slope with some California crudes.  
20% low sulfur, mostly Ardjuna with some  
Alaskan South Slope.

SPECIAL  
REFINING:

This product is salt filtered and clay  
treated. There are no reformates added  
or no hydrogenation. Butanes are added  
for vapor pressure control.

ARCO



DEFENSE LOGISTICS AGENCY  
DEFENSE CONTRACT ADMINISTRATION SERVICES MANAGEMENT AREA SAN ANTONIO  
RESIDENCY OFFICE  
CENTURY BUILDING 11TH FLOOR  
2120 TRAVIS ST.  
HOUSTON, TEXAS 77002

IN REPLY  
REFER TO DCRT-GSQF (G. Michaud/(713)947-2444/ers)

26 October 1981

SUBJECT: Request for JP-4 Fuel Samples

TO: AFVAL/POSF  
ATTN: Maj D. Potter  
Wright-Patterson AFB OH 45433

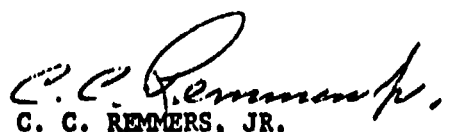
1. Reference your letter dated 16 Sep 81, subject as above.
2. The following information provides some background history of the crude oil used in the production of JP-4 in this area.
  - a. Refinery and location: Friendswood Refining Corp., Friendswood TX.
  - b. Sample submissions were obtained from batch number 66. The attached laboratory test report provides analysis results.
  - c. Crude Sources: The refinery presently procures their crude through Exxon from two oil fields in Texas, i.e., the Giddings Field located near San Antonio, TX and the Saratoga Field located in North Texas.
  - d. Characterization of the Crude:
    - (1) Gravity is approximately 42.0 °API.
    - (2) Aromatic content is approximately 3%.
    - (3) Non-paraffinic.
    - (4) Crude has less than 0.1% wt sulfur.
  - e. Refinery Process:
    - (1) Finished blend is not hydrotreated.
    - (2) Product is clay filtered.
    - (3) No reformates in the finished blend.
    - (4) Finished blend is caustic treated.
    - (5) No butanes or pentanes are used to achieve vapor pressure.

FRIENDSWOOD

DCRT-GSQF                      PAGE 2  
SUBJECT: Request for JP-4 Fuel Samples

3. Hopefully the above will provide you with sufficient data to carry out your research. Should you have further questions, please contact the undersigned or Mr. G. Michaud at AUTOVON: 940-1494.

1 Atch  
Test Report

  
C. C. REMMERS, JR.  
Chief  
Petroleum Operations Branch F

cc: Sanitary Engineering Research Lab  
Attn: Mr. Leon Hunter  
University of California Berkeley

**FRIENDSWOOD REFINING CORP.**  
2502 Choate Rd.  
Houston, Tx. 77210

Item	Method	Appearance	Results	Spec. Limit	Report date	
10	D156	Color (Saybolt)	1.1/1.9	to be reported	10-22-81	
20	--	Visual, Bright & Clear (B&C)	1.8/1.0	B & C	Contract #	
Composition						
100	D3242	Acidity, total (mg KOH/g)	0.1/0	0.15 max.	Order #	
110	D1319	Aromatics (vol. %)	1.2/2	25% max.	Date Sampled 10-22-81	
120	D1319	Olefins (vol. %)	1.1/1	5.0% max.		
130	D3227	Mercaptan Sulphur (wt. %)	0.0/0.5	0.05 max.		
140	D484	Doctor test (pos, neg)	None	report		
150	D1266	Sulphur total (wt. %)	0.1/1.5	0.4 max.	Sampler	
Volatility						
200	D85	Distillation (BP(C))	1.1/3.1	report	Batch # 6.51	
205		10% (C)	2.1/4	"	Tank # 651	
210		20% (C)	2.4/7	145°C max.	Quantity U.S. Gallons	
215		50% (C)	2.9/6	190°C max.		
220		90% (C)	4.1/8	245°C max.		
230		Final BP (C)	4.5/4	270°C max.		
235		Residue (%)	1.0/0	1.5 max.	Sample #	
240		Loss (%)	0.0/0	1.5 max.	Location	
430	D3322	Smoke Pt. mm Min.	2.6/0	20.0 min.	2502 Choate	
270	D1298	Gravity, API (60°F)	3.6/3	45.0-57.0	Rd. Houston	
290	D323	Vapor pressure (lb. Reid)	2.5/3	2.0-3.0 PSI	Product Name	
Fluidity						
300	D2386	Freezing point (C)	-2.1/0	-58°C max.	Destination	
Combustion						
400	D1405	Aniline-Gravity product	4.6/4.2	3250 min.	Grade	
Corrosion						
500	D130	Copper strip-2hr. 212°F	7/1A	1B max.	Remarks--	
Stability						
611	D3241	JFTOT @ 260°C P (mm Hg)	0.5/1	25 mm max.	FRIENDSWOOD	
612	D3241	JFTOT @ 260°C tube color	3/1	less than 73		
Contaminants						
710	D381	Existent Gum (mg/100ml)	0.3/3	7.0 max.		
720	App. A	Particulates (mg/liter)	0.3/5	1.0 max.	1171006	
721	App. A	Filtration time (minutes)	5/5	15 max.		
722	App. A	Vacuum (in/Hg)	7/0	20 min.		
723	App. A	Volume Fuel Filtered (ml)	3.7/8.5	report		
730	D1094	Water Reaction Vol. Change	0.0/0	Report ml.	70045	
740	D1094	Water Reaction		18 min.		
750	D2550	Rotinas Interface-asp.	1/1			
		HSIM	9/9	70 min.		
Additives						
800	FTM-5327	Anti-Ging (vol. %)	1.2/2	100 - 150	Approved By	
810	Antioxidant	(lb/H Bbl)	0.0/0	0 min. - 8.4 max.		
820	Corrosion Inhibitor	(lb/H Bbl)	Arco 4415	3 min. - 8 max.		
830	Metal Deactivator	(lb/H Bbl)	0.0/0			
840	Antistatic	ppm	0.0/0			
Other Tests						
900	D2024	Conductivity (U)				

## APPENDIX B

### CALCULATION OF LC 50 VALUES

LC 50 values and their 95 % confidence limits were calculated from the raw bioassay data by the moving average method. The procedure was based on a paper by Bennett (1952) and is recommended by Stephan (1977) as one of the best all-round methods that can handle a wide range of data, with or without partial kills. The following computer program was written in Basic (Sinclair Extended) to facilitate the computation. The program initially attempts to carry out a 5-term moving average calculation, scanning the data for the appropriate pairs of 5-term averages that span the 50 % kill level. If these requirements are not met the data are rescanned in a 3-term moving average computation. The results are expressed either as percent WSF or ppm (assuming the concentration is known) on request. The computation of the 95 % confidence limits for the LC 50's is based on the variance expression in the paper by Bennett.

```

5  REM "LC50"
10  GOSUB 900
35  PRINT AT 8, 0; "ENTER SAMPLE NAME OR NUMBER"
36  INPUT A$
38  PRINT AT 8, 0; "ENTER CONC. IN PPM. (IF AVAILABLE -
    OTHERWISE ENTER ""0"" )"
40  INPUT B
42  CLS
43  GOSUB 900
45  PRINT AT 8, 0; "NUMBER OF CONTROLS?"
50  INPUT A
52  CLS
53  GOSUB 900
55  PRINT AT 8, 0; "ENTER CONTROL HATCH NUMBERS:"
56  LET TA=0
57  FOR R=1 TO A
58  INPUT CONO
59  LET TA=TA + CONO
60  NEXT R
65  LET TA=TA/A
66  CLS
67  GOSUB 900
70  PRINT AT 8, 0; "ENTER NUMBER OF DILUTIONS"
75  INPUT DILNO
80  DIM D (DILNO+1)
83  DIM H (DILNO+1)
85  DIM X (DILNO+1)
90  PRINT AT 8, 0; "ENTER EACH DILUTION AND HATCH NO. IN ORDER"
92  PAUSE 92
94  CLS
95  PRINT AT 3, 4; "DILUTION"; TAB 18; "HATCH NO."
97  FOR R=8 TO 23
99  PLOT R, 35
101 PLOT R+28, 35
103 NEXT R

```

```

105  FOR R=1 TO DILNO
110  INPUT D(R)
115  INPUT H
120  PRINT AT (5+R), 7; D(R); TAB 21; H
124  REM CALC. UNHATCHED FRACTION
125  LET H(R) = 1-H/TA
126  IF SGN H(R) = -1 THEN LET H(R) = 0
130  NEXT R
135  PRINT AT 16, 0; "IF DATA OK THEN TYPE ""CONT"" AND ""ENTER"".
      IF NOT TYPE ""GOTO 95"" AND ""ENTER""
136  STOP
159  REM DOSE INTERVAL
160  LET DI = D(3) - D(2)
170  IF DILNO >= 6 THEN LET MAN = 4
171  IF DILNO <= 5 THEN LET MAN = 2
210  GOSUB 300
219  REM CALC LC50
220  IF MAN = 2 THEN LET MAN = 3
223  PRINT AT 8, 0; "LC50 AS P.CENT. W(SF) OR P(PM)?"
224  FOR U = 32 TO 33
225  PLOT U, 25
226  PLOT U+18, 25
227  NEXT U
228  INPUT B$
229  CLS
230  LET Q = R
231  LET LC50 = D((Q-1)-(MAN-2)) + C * DI
232  IF B$ = "P" THEN LET LC50 = B * LC50/100
233  LET V = LC50
234  LET I = 9
235  LET J = 22
236  GOSUB 9500
237  IF LOWV > .5 OR HIGHV < .5 THEN PRINT AT I, 0; TAB (J-LEN
      2$+1); "7 spaces"

```



```

238 GOSUB 710
239 PRINT AT 9, 2; A$
240 IF Y = 4 THEN PRINT AT 16, 6; "5"
241 IF Y = 2 AND LOWV < .5 AND HIGHV > .5 THEN PRINT AT 16, 6; "3"
243 PRINT AT 19, 0; "TYPE" "W", "P" OR "S" FOR (1) PC-WSF
    (2) PPM (3) STOP, RESPECTIVELY"
244 REM START CON. LIMITS CALC.
245 IF Y = 4 THEN GOSUB 600
250 IF Y = 2 THEN GOSUB 630
255 GOTO 660
299 REM 5-TERM CALC.
300 LET L = 1
310 LET LOWT = 0
320 LET HIGHT = 0
330 FOR R = L TO MAN + L
240 LET LOWT = LOWT + H(R)
350 LET HIGHT = HIGHT + H(R+1)
360 NEXT R
370 LET LOWV = LOWT/MAN+1)
380 LET HIGHV = HIGHT/(MAN+1)
390 IF LOWV < .5 AND HIGHT > .5 THEN GOTO 440
400 LET L = L+1
410 IF R < DILNO THEN GOTO 310
419 REM 3-TERM CALC.
420 IF MAN = 2 THEN GOTO 440
421 LET MAN = MAN-2
430 GOTO 300
440 LET C = (.5 - LOWV)/(HIGHV - LOWV)
449 REM SET VARIABLES FOR CON. LIMIT CALC.
450 LET K = 1
460 LET Y = MAN
470 FOR S = R - (MAN+1) TO R
480 LET X(K) = (1-H(S)) * H(S)
490 LET K = K+1
500 NEXT S

```

```

510 RETURN
600 LET A = 0
605 FOR R = 2 TO 5
610 LET A = A+X(R)
615 NEXT R
620 RETURN
630 LET A = 0
635 FOR R = 2 TO 3
640 LET A = A+X(R)
645 NEXT R
650 RETURN
660 IF MAN = 4 THEN LET TVAL = 2.57
670 IF MAN = 3 THEN LET TVAL = 3.18
680 LET CL95 = TVAL * SQR (((DI/(HCY+2) - H(1)))**2 * ((A+X(1)) * (1-C)
    ** 2 + (2 * C * (1-C) * A) + (A+X(Y+2)) * C** 2)/TA)/(Y+2))
681 IF B$ = "P" THEN LET CL95 = B + CL95/100
687 LET V = CL95
688 LET I = 9
689 LET J = 29
690 GOSUB 9500
700 IF LOWV > .5 OR HIGHV < .5 THEN PRINT AT I, 0; TAB (J-LEN
    Z$+1); "7 spaces"
701 IF INKEY$ = "" THEN PAUSE 32767
703 IF INKEY$ = "" THEN GOTO 701
704 IF INKEY$ = "S" THEN STOP
705 IF INKEY$ = "W" THEN LET B$ = "W"
706 IF INKEY$ = "P" THEN LET B$ = "P"
707 IF INKEY$ = "W" OR INKEY$ = "P" THEN PRINT AT I, 16; "15 spaces"
708 IF INKEY$ = "W" OR INKEY$ = "P" THEN GOTO 231
709 IF INKEY$ <> "S" OR INKEY$ <> "W" OR INKEY$ <> "P" THEN
    GOTO 701
710 PRINT AT 3.2; "TEST MATERIAL"
712 IF B$ = "W" THEN PRINT AT 3, 18; "PER CENT" "WSF" ""
713 IF B$ = "P" THEN PRINT AT 3, 19; "PTS/MILLION"
715 FOR R = 4 TO 29

```

```

720 PLOT R, 35
725 NEXT R
730 FOR R = 34 TO 61
735 PLOT R, 35
740 NEXT R
745 PRINT AT 5, 23; "95PC. CONF"
750 PRINT AT 6, 18; "LC503spaces LIMITS"
755 FOR R = 35 TO 44
760 PLOT R, 29
765 NEXT R
770 FOR R = 46 TO 63
775 PLOT R, 29
780 NEXT R
785 IF Y = 4 OR Y = 2 THEN PRINT AT 16, 6; " -TERM MOVING
    AVERAGE"
786 IF LOWV < .5 AND HIGHV < .5 THEN PRINT AT 16, 6; "DILUT. RANGE
    TOO LOW"
787 IF LOWV > .5 AND HIGHV > .5 THEN PRINT AT 16, 6; "DILUT. RANGE
    TOO HIGH"
790 FOR R = 12 TO 53
795 PLOT R, 12
796 PLOT R, 9
800 NEXT R
805 FOR R = 9 TO 12
810 PLOT 11, R
815 PLOT 54, R
820 NEXT R
900 PRINT AT 1, 2; "LC50 - MOVING AVERAGE METHOD"
902 FOR R = 3 TO 60
904 PLOT R, 39
906 NEXT R
910 RETURN

```

```

9502 LET U$ = "9999.99"
9505 LET Z$ = ""
9510 LET XL = 0
9515 FOR Z = 1 TO LEN U$
9520 IF XL THEN LET Z$ = Z$ + "0"
9525 IF U$(Z) <> "." THEN GOTO 9535
9530 LET XL = NOT XL
9535 NEXT Z
9540 LET XL = INT ABS V * SGN V
9545 LET XP = INT (ABS (XL-V) * 10** LEN Z$)
9550 IF LEN Z$ THEN GOTO 9565
9555 LET Z$ = STR$ XL
9560 GOTO 9570
9565 LET Z$ = STR$ XL + "." + Z$(1 TO LEN Z$ - LEN STR$ XP) + STR$
XP + Z$(1 TO LEN Z$)
9570 IF LEN Z$ > LEN U$ THEN LET Z$ = Z$(LEN Z$ - LEN U$ + 1 TO)
9575 PRINT AT 1,0; TAB (J - LEN Z$ + 1); Z$;
9580 RETURN

```

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